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BIO 204

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Course code: BIO 204

Course title: Biological Techniques

Course credit load: 2 Units

Series 1: Microscopy and Slide Preparation

- **Part 1.1: The Light Microscope**
  - Sub Part 1.1.1: Parts of the light microscope and their functions
  - Sub Part 1.1.2: Handling and care of the microscope
  - Sub Part 1.1.3: Magnification and resolution
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Introduction

Microscopy is the foundation of biological laboratory work. The ability to observe cells, tissues, and microorganisms depends on understanding how microscopes work and how to prepare

specimens for examination. This Series covers the light microscope and its use, slide preparation techniques, biological drawing conventions, and an introduction to electron microscopy.

Mastery of these practical skills is essential for all subsequent laboratory work in Biological Techniques. By the end of this Series, you will be able to handle and operate a light microscope correctly, prepare and stain slides, produce accurate biological drawings, and distinguish between light and electron microscopy.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- SLO 1.1 identify the parts of a light microscope and state their functions (Linked to Part 1.1),
- SLO 1.2 describe the correct procedures for handling and focusing a light microscope (Linked to Part 1.1),
- SLO 1.3 explain the stages in preparing a temporary wet mount and applying stains (Linked to Part 1.2),
- SLO 1.4 produce accurate biological drawings following accepted conventions (Linked to Part 1.3),
- SLO 1.5 use a hand lens correctly and apply scale and labelling conventions (Linked to Part 1.3),
- SLO 1.6 distinguish between light and electron microscopes and state their applications (Linked to Part 1.4), and
- SLO 1.7 describe the limitations of electron microscopy and the conditions required for specimen preparation (Linked to Part 1.4).

1.1 The Light Microscope

1.1.1 Parts of the light microscope and their functions

The light microscope uses visible light and a system of glass lenses to magnify small objects. Its main structural components are: eyepiece (ocular lens, usually x10; the lens through which the observer looks); body tube (connects eyepiece to the objective lenses); revolving nosepiece

(holds and rotates objective lenses); objective lenses (low power x4 or x10, high power x40, oil immersion x100); stage (flat platform holding the slide); stage clips or a mechanical stage (hold the slide in place); and the base (supports the instrument).

Focusing components: coarse adjustment knob (large knob; moves the stage or body tube for initial focusing); fine adjustment knob (small knob; gives precise focusing); condenser (directs light upward through the specimen); iris diaphragm (controls the amount of light reaching the specimen); and light source (built-in lamp or mirror). Total magnification = eyepiece magnification x objective magnification.

Fill in the blank: Total magnification of a microscope is calculated by multiplying the _____ magnification by the objective lens magnification; for a x10 eyepiece and x40 objective, the total magnification is x400.

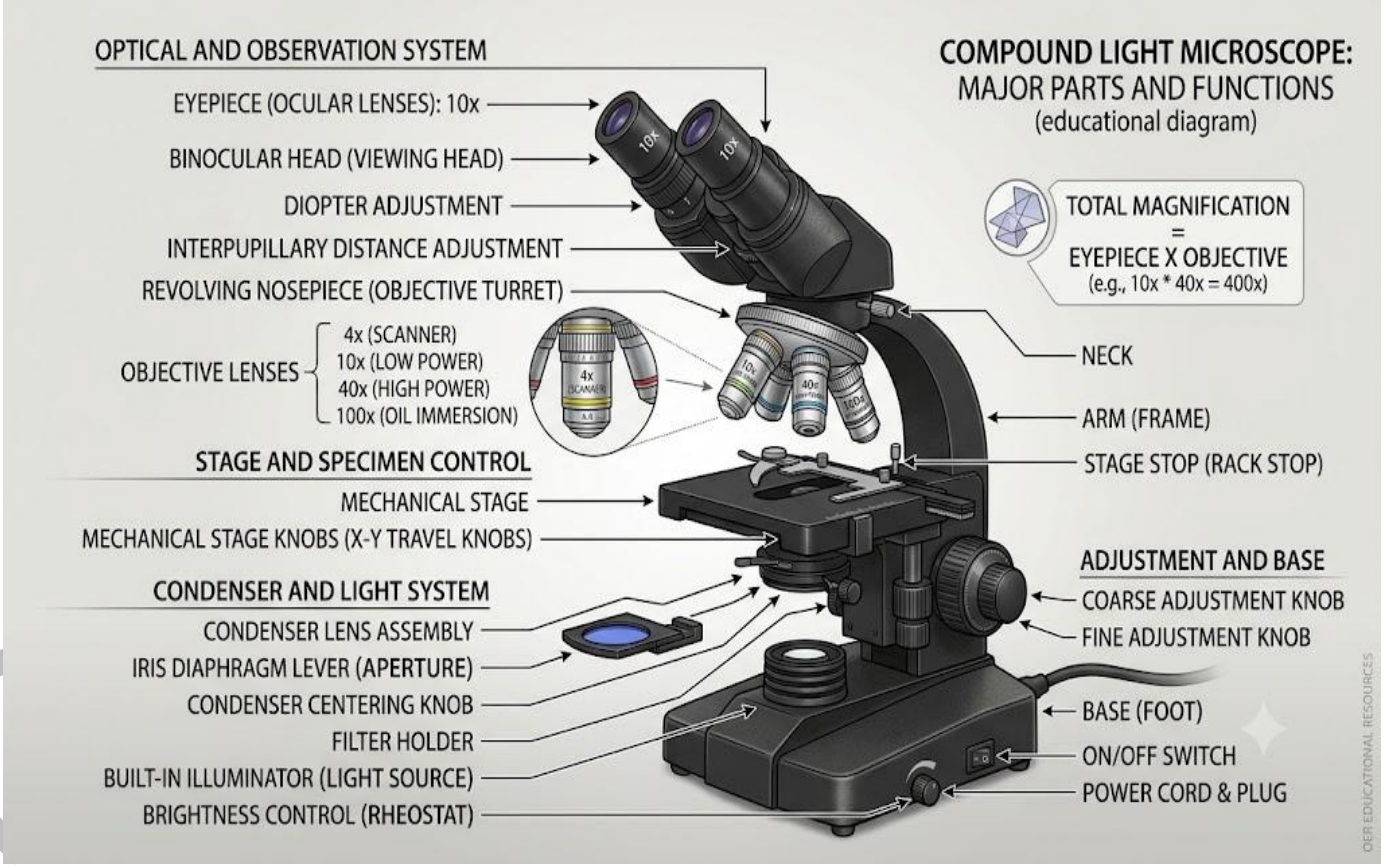

Figure 1.1: Labelled diagram of a compound light microscope

1.1.2 Handling and care of the microscope

Correct handling protects the microscope and ensures reliable results. Always carry the microscope with one hand under the base and the other gripping the arm. Place it on a flat, stable bench away from the bench edge. Begin with the lowest-power objective in position. To focus: place the slide on the stage, lower the objective close to the slide using the coarse knob while looking from the side (not through the eyepiece), then slowly raise the objective while looking through the eyepiece until the image is in focus.

Care and maintenance: clean lenses only with lens tissue (never paper or cloth); never force the coarse adjustment knob; always store the microscope covered with its dust cover; rotate back to the lowest objective before removing a slide; and never tilt the microscope when using water-immersion or oil-immersion objectives. Report damaged parts to the laboratory instructor immediately.

Fill in the blank: When focusing a microscope, always begin with the _____ power objective and use the coarse adjustment knob to bring the objective close to the slide before looking through the eyepiece.

1.1.3 Magnification and resolution

Magnification is the ratio of the image size to the actual size of the object. It tells us how many times larger the image appears compared to the specimen. Resolution (or resolving power) is the ability of the microscope to distinguish two closely spaced points as separate. A microscope may magnify an image many times but still produce a blurry result if its resolution is poor. The limit of resolution of a light microscope is approximately 0.2 micrometres (200 nm).

Resolution depends on the wavelength of light used and the numerical aperture of the objective lens. Shorter wavelengths give better resolution. The formula for actual size is: Actual size = Image size / Magnification. For example, if a cell appears 40 mm wide under x400 magnification, its actual width is 40 mm / 400 = 0.1 mm = 100 micrometres. Understanding this relationship is essential for making accurate measurements from microscope observations.

Fill in the blank: The limit of resolution of a light microscope is approximately _____ micrometres; resolution is the ability to distinguish two closely spaced points as separate structures rather than as one blurred image.

Pilot questions 1.1

1. Name six parts of a light microscope and state the function of each.
2. Describe the correct procedure for carrying and setting up a light microscope.
3. Distinguish between magnification and resolution.
4. A cell measures 20 mm in a micrograph taken at x200. Calculate its actual size in micrometres.
5. State three rules for the proper care of a light microscope.

1.2 Slide Preparation (Microtomy)

1.2.1 Types of microscope slides and mounting media

Microscope slides are flat glass rectangles (approximately 75 x 25 mm) on which specimens are placed for examination. A cover slip (cover glass) is a thin square of glass placed over the specimen to protect the objective lens and flatten the specimen. Temporary slides are prepared fresh and used immediately; permanent slides are preserved with mounting media such as Canada balsam or DPX and can be stored for years.

Mounting media serve to: hold the specimen flat; improve the refractive index match between the specimen and glass (reducing light scatter); preserve the specimen; and seal the cover slip. Aqueous mounting media (e.g. water, glycerol) are used for temporary preparations of living or unfixed material. Resinous media (e.g. DPX, Canada balsam) are used for permanent preparations after dehydration and clearing of the specimen.

Fill in the blank: A _____ slide is prepared fresh and used immediately, while a permanent slide is sealed with resinous mounting media such as DPX and can be stored for long periods.

1.2.2 Preparation of temporary wet mounts

Steps for preparing a temporary wet mount: (1) clean the slide and cover slip with a lint-free cloth; (2) place a small drop of water (or appropriate mounting fluid) at the centre of the slide; (3) place the specimen (e.g. a thin section of onion epidermis or a smear of cheek cells) into the drop; (4) hold the cover slip at 45 degrees and gently lower it onto the drop to exclude air bubbles; (5) remove excess water with filter paper at the edge of the cover slip.

Sectioning: thin sections improve light transmission and image clarity. For soft plant tissue, hand sections are cut with a sharp razor blade; for harder tissue, a microtome (a precision sectioning instrument) is used. Sections should be as thin and uniform as possible. Squash preparations crush soft tissue (e.g. root tip meristems) to spread cells in a single layer; smear preparations spread liquid material (e.g. blood, cheek cells) directly onto the slide.

Fill in the blank: When lowering a cover slip onto a wet mount, hold it at _____ degrees to the slide and lower it slowly to avoid trapping air bubbles beneath the cover slip.

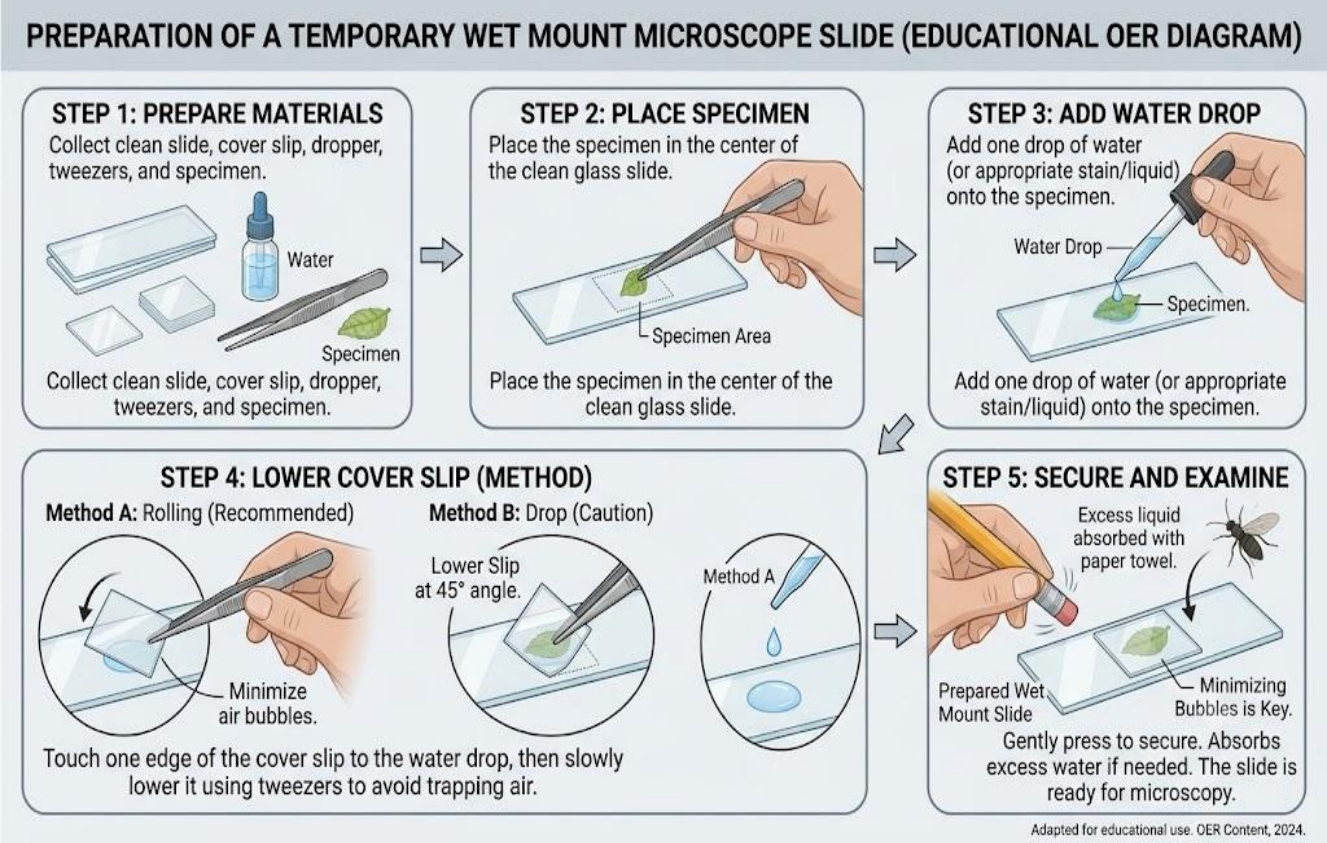

Figure 1.2: Steps in preparing a temporary wet mount

1.2.3 Staining techniques

Stains increase contrast between different cell structures, making them more visible under the microscope. Most biological materials are nearly transparent and difficult to observe without staining. Common stains used in biological techniques: iodine solution (stains starch blue-black; stains nuclei and cytoplasm yellow-brown); methylene blue (stains nuclei blue; used for animal cells); acetocarmine or aceto-orcein (stains chromosomes red; used for mitosis preparations); crystal violet (stains bacterial cell walls in Gram staining).

Staining procedure for a temporary mount: add a small drop of stain at one edge of the cover slip; draw the stain under the cover slip by touching a piece of filter paper to the opposite edge;

allow the stain to diffuse through the specimen for 1 to 2 minutes; rinse off excess stain if necessary. Vital stains (e.g. neutral red, Janus green B) stain living cells without killing them; fixative stains kill and preserve the cell before staining to maintain structure.

Fill in the blank: Iodine solution stains starch _____ and is commonly used to show starch grains in plant cells; it also stains nuclei and cytoplasm yellow-brown.

Pilot questions 1.2

1. Distinguish between temporary and permanent microscope slides.
2. Outline the steps for preparing a temporary wet mount of onion epidermis.
3. State the function of stains in microscopy and give two examples with their uses.
4. Distinguish between a squash preparation and a smear preparation.
5. Explain why thin sections are preferred in microscopy preparations.

1.3 Biological Drawings and Diagrams

1.3.1 Rules and conventions for biological drawings

Biological drawings must be accurate representations of what is actually seen, not artistic impressions. Key rules: use a sharp pencil (HB or 2H) on plain white paper; draw only what you observe (not what you expect to see); use continuous, clean lines without shading or colouring; make the drawing large enough to show detail clearly (occupying at least half the page); do not use cross-hatching or colour to show different regions; and do not use arrows for label lines (use straight ruled lines ending at the structure).

Every biological drawing must include: a title (name of the organism and the type of preparation); magnification (e.g. x400); labels (written in pencil with horizontal label lines leading to the structure; never slanting or crossing each other); and a scale bar where possible. Label lines must not cross each other. Drawings should be made while looking through the microscope, not from memory after removing the slide.

Fill in the blank: In a biological drawing, label lines must be _____ (not slanting) and must not cross each other; the drawing should be made in pencil on plain white paper using clean, continuous lines.

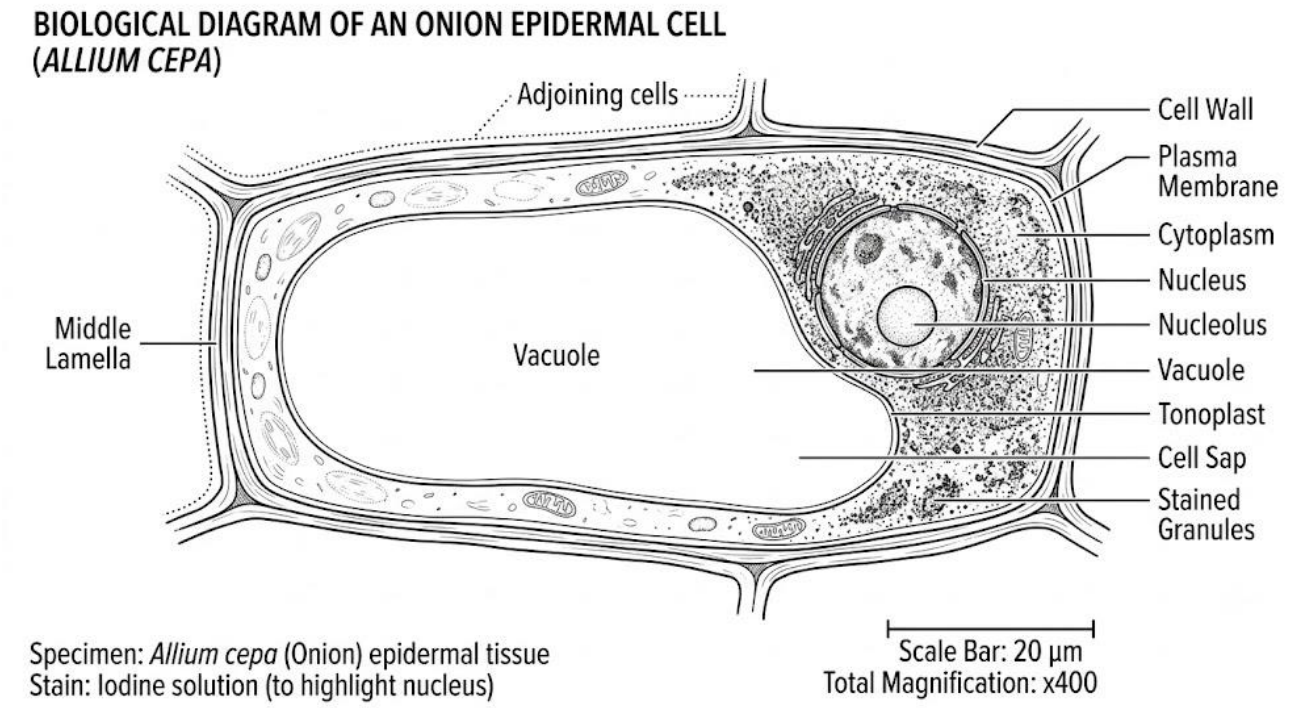

Figure 1.3: Example of a correctly drawn biological diagram of an onion epidermal cell

1.3.2 Use of the hand lens

A hand lens (magnifying glass) is a simple single convex lens typically of x5, x10, or x20 magnification used to examine specimens too small for the naked eye but too large for a compound microscope. To use a hand lens correctly: hold the lens close to the eye (not to the specimen); bring the specimen toward the lens until it comes into focus; maintain adequate lighting (use natural light or a lamp from the side); and keep both eyes open to reduce eyestrain.

The hand lens is used for preliminary examination of organisms and specimens before microscopy, for examining gross morphology of insects or leaves, and for field work where a compound microscope is not available. After examining with a hand lens, a drawing is made following standard biological drawing conventions. The hand lens does not replace the compound microscope for cellular-level observations.

Fill in the blank: To use a hand lens, hold the lens close to the _____ and bring the specimen toward the lens until the image comes into focus; do not hold the lens close to the specimen and look through it from a distance.

1.3.3 Scale, labelling, and annotation

Scale in biological drawings indicates the relationship between the size of the drawing and the actual size of the specimen. A scale bar is a line drawn on the diagram representing a stated actual length (e.g. a line of 20 mm on the drawing labelled "50 micrometres" indicates that 20 mm on paper equals 50 micrometres in reality). Scale bars are preferred over stating magnification alone because they remain accurate even if the drawing is photocopied or resized.

Annotation adds written notes to a drawing to describe function, properties, or observations beyond the label. For example, a label might read "nucleus" while an annotation adds "contains DNA; controls cell activity." Labels identify structures; annotations explain them. Good annotations improve understanding and demonstrate observation skills. All labels and annotations are written in pencil in a biology practical, placed to the right of the drawing where possible, and never overlapping the drawing.

Fill in the blank: A scale bar is preferred over stating magnification alone because it remains accurate even if the drawing is _____ or resized; it shows a line representing a stated actual length on the diagram.

Pilot questions 1.3

1. State five rules for making a correct biological drawing.
2. Explain the correct procedure for using a hand lens.
3. Distinguish between a label and an annotation in a biological drawing.
4. A drawing of a cell measures 60 mm across; the actual cell is 30 micrometres wide. Calculate the magnification and draw a suitable scale bar.
5. Why is a scale bar preferred over stating magnification on a biological drawing?

1.4 The Electron Microscope

1.4.1 Types and principles of electron microscopy

The electron microscope uses a beam of electrons instead of light to form an image. Because electrons have a much shorter wavelength than visible light, the electron microscope has a much higher resolution (approximately 0.2 nanometres), allowing the fine structure of cells (ultrastructure) to be studied. Two main types exist: the Transmission Electron Microscope (TEM) passes electrons through a thin section of specimen; internal cell structures appear in the resulting image.

The Scanning Electron Microscope (SEM) bounces electrons off the surface of a specimen coated with a thin layer of metal (usually gold); it produces three-dimensional surface images. SEM is used to study the external structure of cells, organisms, pollen grains, and insects. TEM is used to study internal ultrastructure such as mitochondrial cristae, nuclear pores, and endoplasmic reticulum. Both types produce black-and-white images; false colour is added digitally for clarity in publications.

Fill in the blank: The Scanning Electron Microscope (SEM) produces _____ surface images of specimens coated with metal, while the Transmission Electron Microscope (TEM) passes electrons through thin sections to reveal internal cell ultrastructure.

1.4.2 Comparison of light and electron microscopes

Key comparisons: radiation used (light microscope uses visible light; electron microscope uses electrons); maximum resolution (light: ~0.2 micrometres; electron: ~0.2 nanometres; electron is ~1,000 times better); maximum useful magnification (light: ~x1,500; electron: ~x500,000); specimen state (light: living or dead; electron: dead, must be fixed and dehydrated); cost (light: low; electron: very high); and image colour (light: natural colour possible; electron: black and white only).

Structural differences: the light microscope focuses light with glass lenses; the electron microscope focuses the electron beam with electromagnetic coils (not glass). Both operate on the

same principle of directing radiation through or onto a specimen and using a lens system to produce a magnified image. The light microscope remains the most widely used instrument in biological laboratories because of its low cost, ease of use, and ability to examine living specimens.

Fill in the blank: The electron microscope has a resolution approximately _____ times better than the light microscope because electrons have a much shorter wavelength than visible light.

Feature	Light Microscope	Electron Microscope
Radiation Type	Visible light (400–700 nm)	Electron beam (~0.005 nm wavelength)
Resolution	~200 nm (limited by light diffraction)	~0.2 nm (1000× better than light)
Magnification	Up to 1,500–2,000×	Up to 1,000,000× (SEM/TEM)
Specimen Requirements	Living or stained specimens; minimal prep	Dead specimens only; extensive prep (fixation, dehydration, coating)
Lenses	Glass lenses	Electromagnetic coils
Image Type	True color	Black and white (false color added later)
Cost	Affordable: ₦80,000–₦5,000,000 ($100–$50,000)	Expensive: ₦40,000,000+ ($50,000–$10,000,000+)

Box 1.1: Comparison of light and electron microscopes

1.4.3 Applications and limitations of electron microscopy

Applications of electron microscopy in biology: studying cell ultrastructure (e.g. internal membrane systems, ribosomes, centrioles); examining viruses too small to resolve with a light microscope; investigating the surface morphology of pollen, spores, and insects (SEM); studying material science and semiconductor surfaces; and diagnosing certain diseases (e.g. identifying virus particles in clinical samples). Electron microscopy has transformed our understanding of cell biology by revealing organelle fine structure.

Limitations: specimens must be dead (killed, fixed in glutaraldehyde, dehydrated, and embedded in resin); the high vacuum inside the microscope prevents examination of living material;

preparation is lengthy and may introduce artefacts (changes to structure caused by the preparation process); the instrument is extremely expensive to purchase and maintain; and specialised training is required to operate it and interpret images. These limitations mean electron microscopy is used in specialist centres, not routine laboratory work.

Fill in the blank: A major limitation of the electron microscope is that specimens must be _____ before examination because the instrument operates under high vacuum, making it impossible to study living cells directly.

Pilot questions 1.4

1. State two types of electron microscope and describe the principle of each.
2. State four differences between a light microscope and an electron microscope.
3. State three applications of electron microscopy in biology.
4. State three limitations of electron microscopy.
5. Explain why electron microscopy cannot be used to study living cells.

Series Summary

This Series introduced the theory and practice of microscopy in biological techniques. The light microscope uses visible light and glass lenses; its parts include the eyepiece, objectives, stage, condenser, and adjustment knobs; total magnification equals eyepiece times objective magnification. Correct handling, focusing from low power, and lens care are essential practical skills. Slide preparation involves selecting the appropriate slide type and mounting medium, preparing thin sections or smears, and applying stains such as iodine or methylene blue to increase contrast.

Biological drawings require pencil on plain paper, continuous lines, horizontal label lines, title, magnification, and scale bars. The hand lens is used for preliminary gross examination; scale bars are preferred over magnification statements. The electron microscope (TEM and SEM) uses

electrons, has far higher resolution (~0.2 nm) and magnification (~x500,000) than a light microscope, but cannot be used on living material, is expensive, and requires specialist preparation. These skills collectively meet SLOs 1.1 to 1.7.

Glossary of Terms

- **Annotation:** written notes added to a drawing to explain function or properties of a labelled structure, beyond simple identification.
- **Artefact:** a structural change introduced into a specimen by the preparation process (fixation, dehydration, sectioning) that is not present in the living organism.
- **Condenser:** a lens beneath the stage of a light microscope that concentrates and directs light upward through the specimen.
- **Cover slip:** a thin square of glass placed over a specimen on a microscope slide to protect the objective lens and flatten the preparation.
- **Electron microscope:** a microscope that uses a beam of electrons rather than light to form a highly magnified, high-resolution image of a specimen.
- **Iris diaphragm:** an adjustable aperture in the condenser that controls the amount of light passing through the specimen.
- **Magnification:** the ratio of the size of an image to the actual size of the object; calculated as image size divided by actual size.
- **Microtome:** a precision instrument used to cut very thin, uniform sections of biological tissue for microscopy.
- **Mounting medium:** a substance used to attach a specimen to a slide and under a cover slip; aqueous media (water, glycerol) are used for temporary mounts; resinous media (DPX, Canada balsam) for permanent mounts.
- **Objective lens:** the lens closest to the specimen in a compound microscope; available in different magnifications (x4, x10, x40, x100 oil immersion).
- **Resolution:** the ability of a microscope to distinguish two closely spaced points as separate; the minimum distance at which two points can still be seen as distinct.
- **Scale bar:** a line drawn on a diagram representing a stated actual length, used to indicate the scale of the drawing independently of any magnification stated.
- **Scanning Electron Microscope (SEM):** an electron microscope that produces three-dimensional surface images by bouncing electrons off a metal-coated specimen surface.

- **Smear preparation:** a microscope slide prepared by spreading a thin layer of liquid material (e.g. blood, cheek cell suspension) directly onto the glass surface.
- **Squash preparation:** a slide prepared by gently crushing soft tissue (e.g. root tip meristem) to spread cells in a single layer for observation of cell division.
- **Transmission Electron Microscope (TEM):** an electron microscope that passes electrons through ultra-thin sections of a specimen to reveal internal ultrastructure at high resolution.
- **Ultrastructure:** the fine internal structure of cells and organelles visible only with an electron microscope; includes details such as mitochondrial cristae, nuclear pores, and ribosome arrangement.
- **Vital stain:** a stain that colours living cells without killing them, allowing observation of cell structures in a living state; examples include neutral red and Janus green B.
- **Wet mount:** a temporary microscope preparation in which the specimen is placed in a drop of water (or other aqueous medium) on a slide and covered with a cover slip.

Concept Check Questions [Series 1]

Objective Questions

1. Total magnification of a microscope equals the _____ magnification multiplied by the objective lens magnification. (Linked to SLO 1.1)
2. The limit of resolution of a light microscope is approximately _____ micrometres. (Linked to SLO 1.1)
3. Iodine solution stains starch _____ and is used to identify starch granules in plant cells. (Linked to SLO 1.3)
4. In biological drawings, label lines must be _____ and must not cross each other. (Linked to SLO 1.4)
5. The electron microscope cannot be used to study living material because it operates under high _____. (Linked to SLO 1.7)

Short-Answer Questions

6. Name five parts of a light microscope and state the function of each. (Linked to SLO 1.1)
7. Outline the steps for preparing a temporary wet mount. (Linked to SLO 1.3)
8. State four rules for biological drawings and explain the purpose of a scale bar. (Linked to SLO 1.4)

9. State three differences between TEM and SEM. (Linked to SLO 1.6)
10. State three limitations of the electron microscope compared to the light microscope. (Linked to SLO 1.7)

Long-Answer Questions

11. Describe the structure and function of the main parts of a compound light microscope, and explain the principles of magnification and resolution. (Linked to SLOs 1.1, 1.2)
12. Describe the preparation of a temporary stained wet mount of onion epidermis and explain the rules for making and labelling a biological drawing from the slide. (Linked to SLOs 1.3, 1.4, 1.5)
13. Compare the light microscope and the electron microscope (TEM and SEM) in terms of principle, resolution, magnification, specimen requirements, and applications; state the limitations of electron microscopy. (Linked to SLOs 1.6, 1.7)

Answers to Concept Check Questions [Series 1]

Objective Questions

1. Eyepiece (ocular).
2. 0.2 micrometres.
3. Blue-black.
4. Horizontal.
5. Vacuum.

Short-Answer Questions

6. Eyepiece (x10; magnifies image from objective); objective lens (x4/x10/x40/x100; primary magnification); stage (holds slide); condenser (focuses light on specimen); coarse adjustment knob (initial focusing); fine adjustment knob (precise focusing).
7. Clean slide; place drop of water at centre; add thin section of onion epidermis; lower cover slip at 45 degrees to exclude air bubbles; draw off excess water with filter paper; add stain (iodine) at one edge and draw under cover slip with filter paper.
8. Rules: use sharp pencil; draw only what is observed; use continuous lines (no shading); draw large; use horizontal label lines that do not cross; include title and magnification. Scale bar

purpose: shows actual length on the drawing; remains accurate when a drawing is photocopied or resized, unlike a stated magnification.

9. TEM passes electrons through thin sections; shows internal ultrastructure; two-dimensional images. SEM bounces electrons off metal-coated surface; shows external three-dimensional surface morphology; requires metal coating of specimen.
10. Cannot examine living material (high vacuum kills cells); specimens require lengthy preparation (fixation, dehydration, embedding); preparation may introduce artefacts; instrument is very expensive; specialised training needed to operate and interpret images.

Long-Answer Questions

11. Parts and functions: eyepiece (x10; magnifies image formed by objective); body tube (connects eyepiece to objectives); revolving nosepiece (rotates to change objectives); objective lenses (x4, x10, x40, x100; primary magnification; closest to specimen); stage (holds slide); stage clips (secure slide); condenser (focuses light on specimen); iris diaphragm (controls light intensity); coarse adjustment knob (initial focus; moves stage up/down); fine adjustment knob (precise focus); light source (illuminates specimen). Total magnification = eyepiece x objective. Magnification: ratio of image size to actual size; image size = actual size x magnification; actual size = image size / magnification. Resolution: ability to distinguish two closely spaced points as separate; light microscope limit ~0.2 micrometres; depends on wavelength of light and numerical aperture of objective; shorter wavelength = better resolution.
12. Wet mount preparation: clean slide and cover slip; place drop of water at centre; peel thin section of onion epidermis using forceps; place in water drop; lower cover slip at 45 degrees to exclude air bubbles; remove excess water with filter paper; apply iodine stain by capillary action using filter paper. Biological drawing rules: use sharp HB pencil on plain paper; draw only what is observed; use continuous clean lines; make drawing large (half page minimum); no shading or colour; horizontal label lines that do not cross; include title, magnification, and scale bar. Scale bar: draw a line of known length on the diagram and label with the actual distance it represents; more reliable than magnification statement alone because it does not change if the image is resized.
13. Light microscope: uses visible light; glass lenses; resolution ~0.2 micrometres; max magnification ~x1,500; can examine living or dead specimens; colour images possible; low cost; routine laboratory use. Electron microscope: uses electron beam; electromagnetic coils; resolution ~0.2 nm (1,000x better than light); max magnification ~x500,000; dead specimens only (fixed,

dehydrated, embedded); black-and-white images (false colour added digitally); very high cost. TEM: electrons pass through thin sections; reveals internal ultrastructure; two-dimensional image. SEM: electrons bounce off metal-coated surface; reveals three-dimensional surface morphology. Limitations of electron microscopy: cannot examine living cells (high vacuum); preparation introduces possible artefacts; very expensive; requires specialist training; lengthy specimen preparation.

Answers to Pilot Questions [Series 1]

Part 1.1

1. Eyepiece (magnifies image from objective); objective lens (primary magnification; closest to specimen); stage (holds slide for examination); condenser (focuses light on specimen from below); coarse adjustment knob (moves stage for initial focus); fine adjustment knob (fine precise focus); iris diaphragm (controls light intensity entering condenser).
2. Carry with one hand under base and one on arm. Place on flat bench. Rotate lowest-power objective into position. Place slide on stage and secure with clips. Looking from the side, lower objective close to slide with coarse knob. Look through eyepiece and raise objective slowly until image appears. Use fine knob to sharpen focus.
3. Magnification is the number of times an image is enlarged compared to the actual object; it equals image size / actual size. Resolution is the ability to distinguish two closely spaced points as separate structures; high magnification with poor resolution produces a large but blurry image.
4. Actual size = image size / magnification = 20 mm / 200 = 0.1 mm = 100 micrometres.
5. Clean lenses only with lens tissue; never force the coarse adjustment knob; store covered with a dust cover; always return to the lowest objective before removing a slide.

Part 1.2

1. Temporary slides are prepared fresh, use aqueous mounting media, and are viewed immediately; they cannot be stored. Permanent slides are fixed, dehydrated, cleared, and mounted in resinous media (e.g. DPX); they can be stored and re-examined for years.
2. Clean slide and cover slip; place a drop of water at the slide centre; peel a single layer of onion epidermis with forceps and place in the water drop; lower cover slip at 45 degrees to exclude air bubbles; remove excess water with filter paper; draw iodine stain under the cover slip from one edge using filter paper at the opposite edge.

3. Stains increase contrast between cell structures that are nearly transparent, making them visible. Iodine: stains starch blue-black; used to reveal starch grains and nuclei in plant cells. Methylene blue: stains nuclei blue; used for animal cells such as cheek cells.
4. Squash: soft tissue (e.g. root tip) is gently crushed on a slide to spread cells in a single layer; used for viewing mitosis. Smear: liquid material (e.g. blood, cheek cells) is spread directly across the slide surface in a thin film; used for blood cell counts and oral cytology.
5. Thin sections allow more light to pass through, improving image brightness and contrast; they reduce overlapping of structures, making individual cells easier to distinguish; they allow higher-power objectives to focus clearly on the specimen without excessive depth of field problems.

Part 1.3

1. Use a sharp HB pencil; draw only what is observed; use continuous clean lines (no shading); make the drawing large; use horizontal label lines that do not cross each other; include a title, magnification, and scale bar.
2. Hold the lens close to the eye (not to the specimen); bring the specimen toward the lens until the image is in focus; maintain good side lighting; keep both eyes open to reduce eye strain.
3. A label identifies a structure (e.g. "nucleus"). An annotation adds explanatory information about function or properties (e.g. "nucleus: contains DNA; controls cell activity"). Labels name; annotations explain.
4. Magnification = image size / actual size = 60 mm / 0.03 mm = x2,000. Scale bar: draw a line of 20 mm on the diagram and label it "10 micrometres" (since 20 mm / 2,000 = 0.01 mm = 10 micrometres).
5. A scale bar remains accurate when the drawing is photocopied or resized because it is part of the image; a stated magnification becomes inaccurate as soon as the image is enlarged or reduced by copying.

Part 1.4

1. TEM: electrons pass through ultra-thin sections of fixed, stained specimen; produces two-dimensional images of internal cell ultrastructure; resolution ~0.2 nm. SEM: electrons bounce off surface of metal-coated specimen; produces three-dimensional surface images; used for external morphology of cells, pollen, and insects.

2. Radiation (visible light vs electrons); resolution (~0.2 micrometres vs ~0.2 nm); maximum magnification (~x1,500 vs ~x500,000); specimen (living or dead vs dead only); lenses (glass vs electromagnetic coils); cost (low vs very high).
3. Studying cell ultrastructure (e.g. mitochondrial cristae, nuclear pores); examining viruses (too small for light microscopes); investigating surface morphology of pollen, spores, and insects (SEM); diagnosing viral infections in clinical samples.
4. Cannot examine living material; lengthy and complex specimen preparation (fixation, dehydration, embedding, sectioning); preparation may introduce artefacts; very expensive to purchase and maintain; requires specialised technical training.
5. The electron microscope operates under high vacuum to prevent electrons scattering off air molecules; biological specimens contain water which would evaporate under vacuum and disrupt cell structure; specimens must therefore be fixed, dehydrated, and embedded before examination, making it impossible to observe living cells.

References and Attributions [Series 1]

Textual References (Books and External Sources)

- Bancroft, J. D., & Gamble, M. (2008). Theory and practice of histological techniques (6th ed.). Churchill Livingstone.
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- Karp, G. (2013). Cell and molecular biology: Concepts and experiments (7th ed.). Wiley.
- Raven, P. H., Johnson, G. B., Mason, K. A., Losos, J. B., & Singer, S. R. (2014). Biology (10th ed.). McGraw-Hill.

Figures, Plates, Tables, and Boxes

- Figure 1.1: Labelled diagram of a compound light microscope Attribution: AI generated. Suggested OER search string: "compound light microscope labelled diagram parts functions OER".

- Figure 1.2: Steps in preparing a temporary wet mount Attribution: AI generated. Suggested OER search string: "temporary wet mount preparation steps microscope slide cover slip OER diagram".
- Figure 1.3: Example of a correctly drawn biological diagram of an onion epidermal cell Attribution: AI generated. Suggested OER search string: "biological drawing onion epidermal cell labelled diagram microscopy conventions OER".
- Box 1.1: Comparison of light and electron microscopes Attribution: AI generated. Suggested OER search string: "light microscope electron microscope comparison table resolution magnification OER".

Course code: BIO 204

Course title: Biological Techniques

Course credit load: 2 Units

Series 2: Principles of Analytical Techniques

- **Part 2.1: Spectrophotometry and Colorimetry**
    - Sub Part 2.1.1: Principles of spectrophotometry
    - Sub Part 2.1.2: Colorimetry and photometry
    - Sub Part 2.1.3: Polarimetry and refractometry
- **Part 2.2: Chromatography**
    - Sub Part 2.2.1: Principles and types of chromatography
    - Sub Part 2.2.2: Paper and thin-layer chromatography
    - Sub Part 2.2.3: Column and gas chromatography
- **Part 2.3: Melting Points and Colligative Properties**
    - Sub Part 2.3.1: Melting point determination
    - Sub Part 2.3.2: Colligative properties of solutions
    - Sub Part 2.3.3: Applications of colligative properties in biology
- **Part 2.4: Centrifugation and Electrophoresis**
    - Sub Part 2.4.1: Principles and types of centrifugation
    - Sub Part 2.4.2: Principles and procedure of electrophoresis
    - Sub Part 2.4.3: Applications of centrifugation and electrophoresis

Introduction

Analytical techniques are laboratory methods used to identify, separate, or quantify the components of biological materials. Spectrophotometry measures light absorbed by solutions to

determine concentration; chromatography separates mixtures based on differential migration through a medium; melting point analysis identifies pure substances; and centrifugation and electrophoresis separate molecules by size, density, or charge. Mastery of these techniques is foundational to all experimental biology.

This Series surveys the principles and practical applications of the major analytical techniques covered in BIO 204. The techniques range from optical methods (spectrophotometry, colorimetry, polarimetry, refractometry) to separation methods (chromatography, centrifugation, electrophoresis) and physical property measurements (melting points, colligative properties). These tools underpin biochemical, microbiological, and ecological research.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- SLO 2.1 explain the principle of spectrophotometry and describe how a spectrophotometer is used to determine concentration (Linked to Part 2.1),
- SLO 2.2 distinguish between colorimetry, photometry, polarimetry, and refractometry and state an application of each (Linked to Part 2.1),
- SLO 2.3 describe the principle of chromatography and compare paper, thin-layer, column, and gas chromatography (Linked to Part 2.2),
- SLO 2.4 explain the Rf value and interpret a chromatogram (Linked to Part 2.2),
- SLO 2.5 explain melting point determination and state four colligative properties of solutions (Linked to Part 2.3),
- SLO 2.6 describe the principle of centrifugation and distinguish between differential and density-gradient centrifugation (Linked to Part 2.4), and
- SLO 2.7 explain the principle of gel electrophoresis and state its applications in biology (Linked to Part 2.4).

2.1 Spectrophotometry and Colorimetry

2.1.1 Principles of spectrophotometry

Spectrophotometry measures the amount of light absorbed by a solution at a specific wavelength. The underlying principle is Beer-Lambert Law: absorbance (A) is directly proportional to the concentration (c) of the absorbing substance and the path length (l) of the light through the solution: A = ecl, where e is the molar absorption coefficient. A spectrophotometer directs a monochromatic beam of light through a cuvette containing the sample and measures how much light reaches the detector on the other side.

Components of a spectrophotometer: light source (tungsten lamp for visible range; deuterium lamp for UV); monochromator (prism or diffraction grating to select a specific wavelength); sample cuvette (quartz for UV, glass or plastic for visible); detector (photodiode or photomultiplier tube); and readout device. Procedure: set the wavelength of maximum absorbance; zero the instrument with a blank (solvent only); place the sample; record absorbance; calculate concentration using a calibration curve (absorbance plotted against known concentrations).

Fill in the blank: Beer-Lambert Law states that absorbance is directly proportional to the _____ of the absorbing substance and the path length of the light beam through the solution.

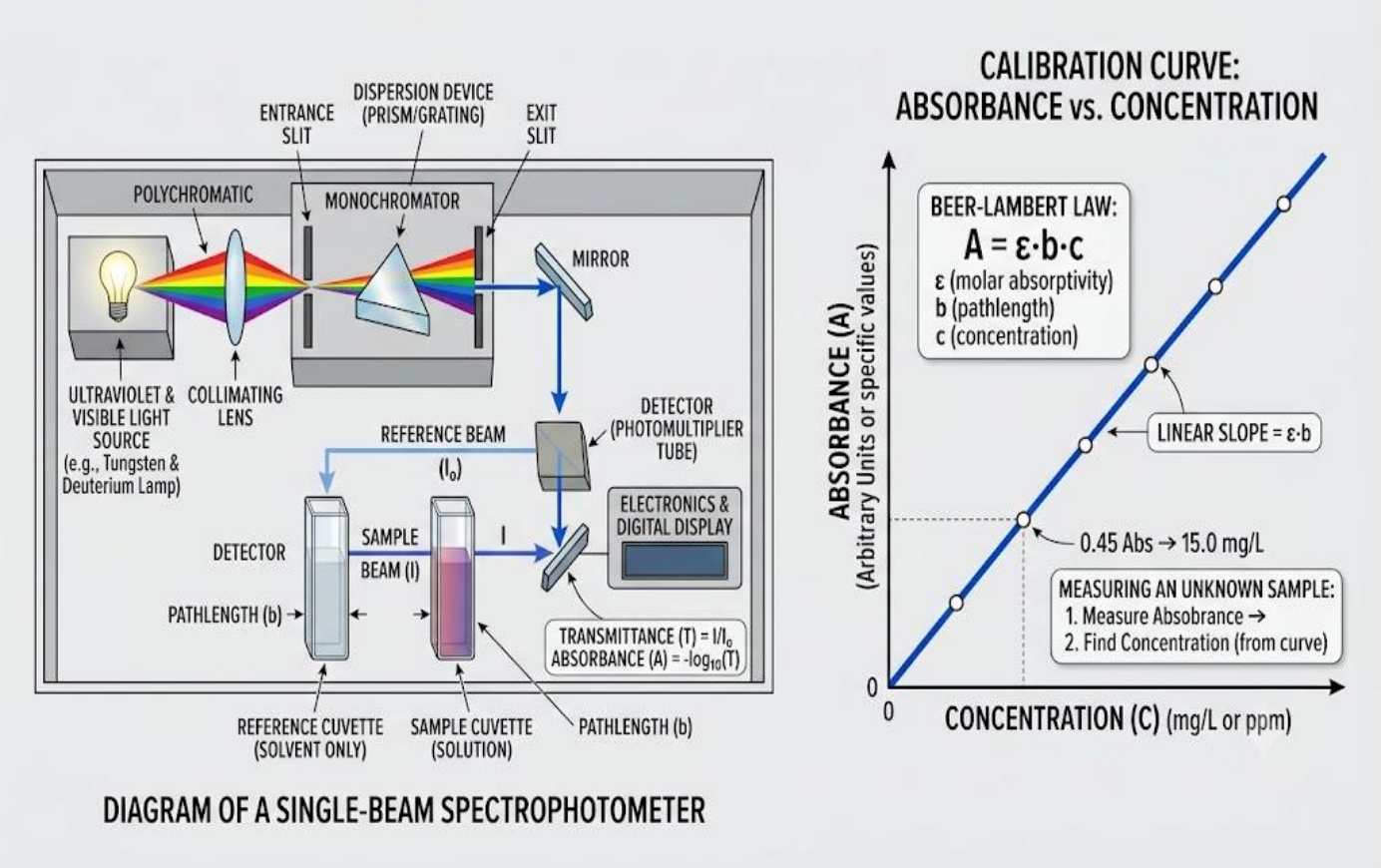

Figure 2.1: Diagram of a spectrophotometer and a Beer-Lambert calibration curve

2.1.2 Colorimetry and photometry

Colorimetry measures the absorbance of visible light by a coloured solution to determine the concentration of a coloured substance. It operates on the same Beer-Lambert principle as spectrophotometry but uses simple coloured filters rather than a monochromator to select a broad waveband. A colorimeter is less precise than a spectrophotometer but is cheaper, more robust, and suitable for routine quantitative work such as measuring glucose, protein, or haemoglobin concentrations in clinical and ecological samples.

Photometry is the broader measurement of light intensity in a beam; it includes colorimetry and spectrophotometry. Flame photometry measures the emission of light by metal ions (e.g. sodium, potassium) when aspirated into a flame; it is used in clinical chemistry to determine electrolyte concentrations in blood serum and urine. In all optical methods, the blank (reagent without the

analyte) corrects for background absorbance; the reference (known standard) establishes the calibration curve against which unknown samples are measured.

Fill in the blank: A colorimeter uses coloured _____ to select a broad waveband of visible light, making it less precise but cheaper and more robust than a spectrophotometer; it is used in routine measurement of coloured solutions such as glucose and haemoglobin.

2.1.3 Polarimetry and refractometry

Polarimetry measures the rotation of plane-polarised light by optically active (chiral) substances. Optically active molecules such as sugars, amino acids, and many drugs rotate the plane of polarised light either clockwise (dextrorotatory, +) or anticlockwise (laevorotatory, -). A polarimeter consists of a light source, a polariser, a sample tube, an analyser (second polariser), and a detector. The angle of rotation (optical rotation) is proportional to concentration; polarimetry is used in the sugar industry to determine sucrose concentration and in the pharmaceutical industry for purity testing.

Refractometry measures the refractive index of a liquid, which is the ratio of the speed of light in a vacuum to its speed in the substance. The refractive index of a solution increases with solute concentration. A refractometer is used to determine the concentration of dissolved solids (e.g. sugar, salt, protein) in solutions; in ecology it measures salinity of water samples; in the food industry it assesses the sugar content of fruit juices and honey; and clinically it estimates urine specific gravity and total serum protein.

Fill in the blank: The _____ index of a solution increases with solute concentration; a refractometer measures this property and is used to estimate the concentration of dissolved solids in solutions such as fruit juice, urine, and seawater.

Pilot questions 2.1

6. State Beer-Lambert Law and name the components of a spectrophotometer.
7. Distinguish between a spectrophotometer and a colorimeter.
8. Explain what a calibration curve is and how it is used in spectrophotometry.
9. State two applications each of polarimetry and refractometry.

10. Explain the principle of flame photometry and give one biological application.

2.2 Chromatography

2.2.1 Principles and types of chromatography

Chromatography separates the components of a mixture based on their differential rates of migration through a stationary phase when carried by a mobile phase. The stationary phase is a solid or liquid held in place (e.g. paper, silica gel, a packed column); the mobile phase is a liquid or gas that moves through or over the stationary phase carrying the mixture. Components that interact strongly with the stationary phase move slowly; those that interact weakly move quickly. This differential migration produces separation.

Key concept: the Rf value (retardation factor) describes the position of a separated spot relative to the solvent front. Rf = distance travelled by the spot / distance travelled by the solvent front. Rf values are characteristic of a compound under defined conditions (solvent system, temperature, stationary phase) and can be used to identify unknown substances by comparison with known standards run under the same conditions. Rf values range from 0 (no migration) to 1.0 (complete migration with the solvent).

Fill in the blank: The Rf value is calculated by dividing the distance travelled by the _____ by the distance travelled by the solvent front; it is a characteristic property used to identify compounds under defined conditions.

2.2.2 Paper and thin-layer chromatography

Paper chromatography (PC) uses chromatography paper as the stationary phase; water held in the cellulose fibres acts as a liquid stationary phase. The mobile phase is an organic solvent or solvent mixture. The mixture to be separated is spotted near the bottom of the paper; the paper is suspended with the bottom edge dipping into the solvent; capillary action draws the solvent up through the paper, carrying the components with it at different rates. PC is used to separate amino acids, sugars, plant pigments, and water-soluble dyes.

Thin-layer chromatography (TLC) uses a thin layer of silica gel or alumina coated on a glass, aluminium, or plastic plate as the stationary phase; the mobile phase is an organic solvent. TLC is faster, more reproducible, and gives sharper spots than PC. After development, colourless spots are visualised under UV light or by spraying with a locating agent (e.g. ninhydrin for amino acids; iodine vapour for lipids). Two-dimensional chromatography runs the chromatogram in one direction, then turns it 90 degrees and runs again in a different solvent to improve separation of complex mixtures.

Fill in the blank: In paper chromatography, capillary action draws the _____ up through the paper, carrying the mixture components at different rates; components with greater affinity for the stationary phase travel more slowly and have lower Rf values.

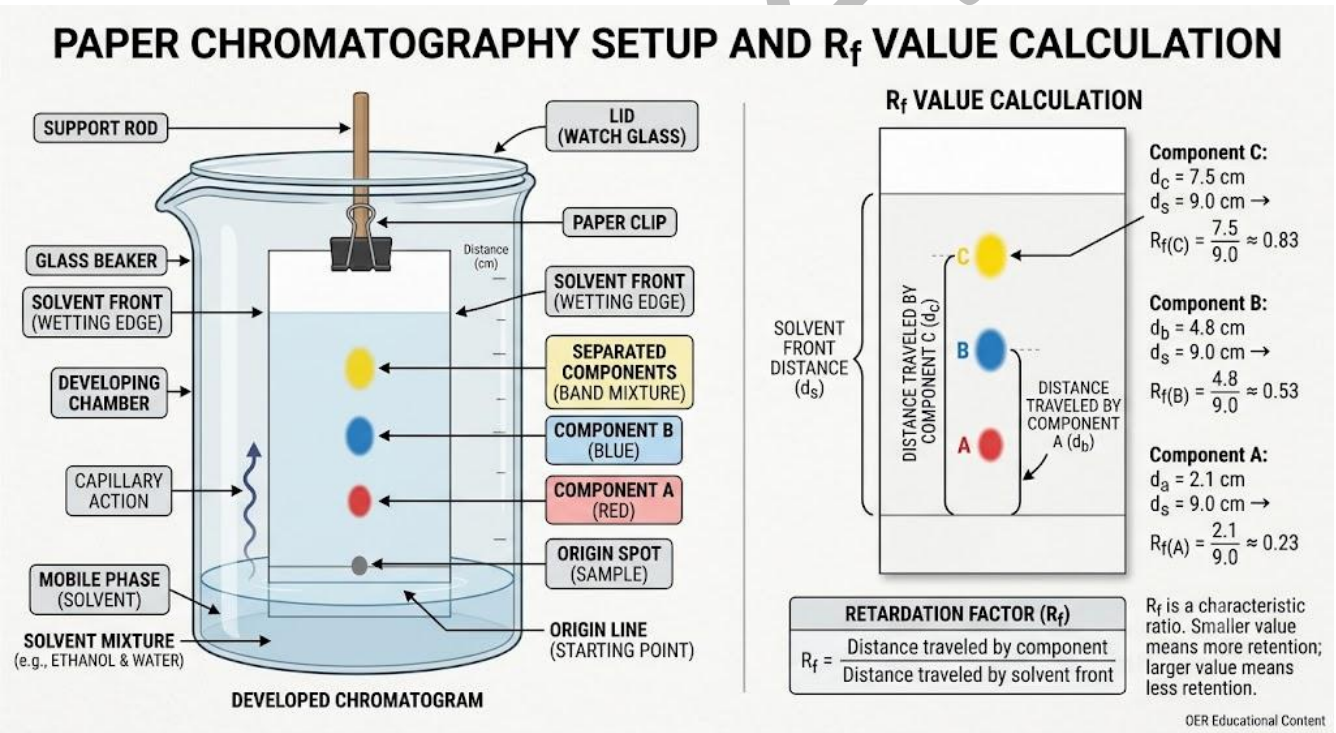

Figure 2.2: Paper chromatography setup and Rf value calculation

2.2.3 Column and gas chromatography

Column chromatography uses a glass column packed with a granular stationary phase (e.g. silica gel, alumina, or ion-exchange resin). The mixture is applied to the top of the column; solvent (mobile phase) is then poured through and the components elute (emerge) from the bottom at

different times. Column chromatography is used preparatively to isolate pure compounds (e.g. isolating a specific protein from a cell extract); it can handle larger quantities than TLC or PC. The order of elution depends on the affinity of each component for the stationary phase.

Gas chromatography (GC) uses an inert carrier gas (e.g. nitrogen, helium) as the mobile phase and a column coated with a liquid stationary phase held at elevated temperature. The sample is vaporised and carried through the column; components separate according to their volatility and affinity for the stationary phase. A detector (e.g. flame ionisation detector) records each component as it elutes; the result is a chromatogram of peaks against time. GC is used to analyse volatile compounds such as fatty acids, steroids, environmental pollutants, and essential oils.

Fill in the blank: In gas chromatography, an inert carrier _____ acts as the mobile phase; the sample is vaporised and components separate according to their volatility and interaction with the liquid stationary phase coating the column.

Pilot questions 2.2

6. State the principle of chromatography and define the terms stationary phase and mobile phase.
7. A chromatogram shows spots at 4.2 cm and 2.6 cm from the baseline; the solvent front is 7.0 cm. Calculate the Rf value of each spot.
8. Distinguish between paper chromatography and thin-layer chromatography.
9. State two differences between column chromatography and gas chromatography.
10. State two biological applications each of column chromatography and gas chromatography.

2.3 Melting Points and Colligative Properties

2.3.1 Melting point determination

The melting point of a pure solid substance is the temperature at which it changes from solid to liquid at atmospheric pressure. It is a characteristic physical property: each pure compound has a sharp, well-defined melting point. Impurities lower the melting point and broaden the melting range. Melting point determination is therefore used to: (1) identify an unknown solid by

comparing its melting point with literature values; (2) assess the purity of a compound (a pure compound melts sharply over a narrow range, typically 0.5 to 1 degree; an impure compound melts over a wide range at a lower temperature).

Procedure using a melting point apparatus: pack a small amount of the dry powdered sample into a capillary tube sealed at one end; place the tube in the melting point block or oil bath; heat slowly (1 to 2 degrees per minute near the expected melting point); observe the temperature at which the sample first begins to soften and the temperature at which it becomes completely liquid; record as the melting range. A digital melting point apparatus with an optical sensor replaces the manual approach for greater reproducibility in modern laboratories.

Fill in the blank: Impurities _____ the melting point of a solid and broaden the melting range; a pure compound melts sharply over a narrow range of approximately 0.5 to 1 degree Celsius.

2.3.2 Colligative properties of solutions

Colligative properties are properties of solutions that depend on the number of solute particles dissolved per unit volume of solvent, not on the chemical nature of the solute. The four colligative properties are: (1) vapour pressure lowering (the presence of a solute reduces the vapour pressure of the solvent above the solution); (2) boiling point elevation (a solute raises the boiling point of the solvent; the elevation is proportional to the molal concentration of solute particles); (3) freezing point depression (a solute lowers the freezing point of the solvent; the depression is proportional to molal concentration); and (4) osmotic pressure (the pressure required to prevent osmosis across a semipermeable membrane separating the solution from pure solvent).

The equations: boiling point elevation = Kb x m (Kb = ebullioscopic constant; m = molality); freezing point depression = Kf x m (Kf = cryoscopic constant). These relationships apply to all solutes regardless of their chemical identity. Electrolytes (e.g. NaCl) that dissociate into ions contribute more particles per formula unit than non-electrolytes (e.g. glucose) and therefore cause greater colligative effects at the same molar concentration.

Fill in the blank: The four colligative properties of solutions are vapour pressure lowering, boiling point elevation, freezing point depression, and _____ pressure; they all depend on the number of solute particles per unit volume rather than their chemical identity.

Property	Effect	Equation	Example
Boiling Point Elevation	Solution boils at a higher temperature than pure solvent	$\Delta T_b = K_b \cdot m$ (where K_b = ebullioscopic constant, m = molality)	Adding salt to water raises boiling point (used in cooking pasta)
Freezing Point Depression	Solution freezes at a lower temperature than pure solvent	$\Delta T_f = K_f \cdot m$ (where K_f = cryoscopic constant)	Salt spread on icy roads lowers freezing point, melting ice
Osmotic Pressure	Pressure required to stop osmosis across a semipermeable membrane	$\pi = M \cdot R \cdot T$ (where M = molarity, R = gas constant, T = temperature)	Movement of water into plant roots; IV fluids in medicine
Vapor Pressure Lowering	Solution has lower vapor pressure than pure solvent	$P = X_{solvent} \cdot P^0_{solvent}$ (Raoult's Law)	Sugar or salt solutions evaporate more slowly than pure water

Box 2.1: Summary of colligative properties and their biological relevance

2.3.3 Applications of colligative properties in biology

Osmotic pressure is the most biologically important colligative property. It governs the movement of water across cell membranes by osmosis: water moves from a region of low solute concentration (low osmotic pressure; high water potential) to a region of high solute concentration (high osmotic pressure; low water potential) across a semipermeable membrane. This principle explains turgor pressure in plant cells, plasmolysis, crenation of red blood cells in hypertonic solutions, and haemolysis in hypotonic solutions.

Freezing point depression is exploited in cryobiology: cells and tissues are preserved at low temperatures in solutions containing cryoprotectants (e.g. glycerol, DMSO) that lower the freezing point and prevent ice crystal formation. Clinically, the freezing point depression of blood plasma (osmometry) measures total osmolality of body fluids (reference range: 275 to 295

mOsm/kg), used to assess dehydration, diabetes insipidus, and electrolyte disorders. Boiling point elevation is used in sterilisation (autoclaving at 121 degrees under pressure ensures temperatures above 100 degrees, killing all spores).

Fill in the blank: Intravenous fluids used in hospitals are designed to be _____ (having the same osmotic pressure as blood plasma) to prevent osmotic damage to red blood cells during administration.

Pilot questions 2.3

6. Explain how melting point is used to assess the purity of a compound.
7. State the four colligative properties of solutions and explain what they have in common.
8. Explain why a 1 mol/L NaCl solution causes a greater freezing point depression than a 1 mol/L glucose solution.
9. Explain the importance of osmotic pressure in the design of intravenous fluids.
10. State two biological applications of freezing point depression.

2.4 Centrifugation and Electrophoresis

2.4.1 Principles and types of centrifugation

Centrifugation separates particles or molecules in a liquid by applying centrifugal force generated by spinning the sample at high speed. The centrifugal force (expressed as relative centrifugal force, RCF, in units of g) causes denser or larger particles to sediment faster than lighter or smaller ones. Speed is expressed in revolutions per minute (rpm) or as RCF: RCF = 1.12 x r x (rpm/1000)^2, where r is the rotor radius in millimetres. A centrifuge consists of a motor, a rotor (holds tubes at fixed or swing-out angles), and a refrigerated chamber in preparative centrifuges.

Types: low-speed centrifugation (up to 5,000 g; sediments cells, cell debris, and precipitates); high-speed centrifugation (up to 100,000 g; sediments mitochondria, chloroplasts, and bacteria); ultracentrifugation (above 100,000 g; sediments ribosomes, large macromolecules, viruses).

Differential centrifugation uses successive increases in speed to pellet different cell fractions sequentially: nuclei first (600 g), then mitochondria (10,000 g), then ribosomes and microsomes (100,000 g). Density-gradient centrifugation (e.g. sucrose or CsCl gradients) separates particles by both size and density, giving higher resolution than differential centrifugation.

Fill in the blank: In differential centrifugation, cell fractions are separated by applying _____ increases in centrifugal force; nuclei sediment at the lowest speed (~600 g) and ribosomes require ultracentrifugation (~100,000 g).

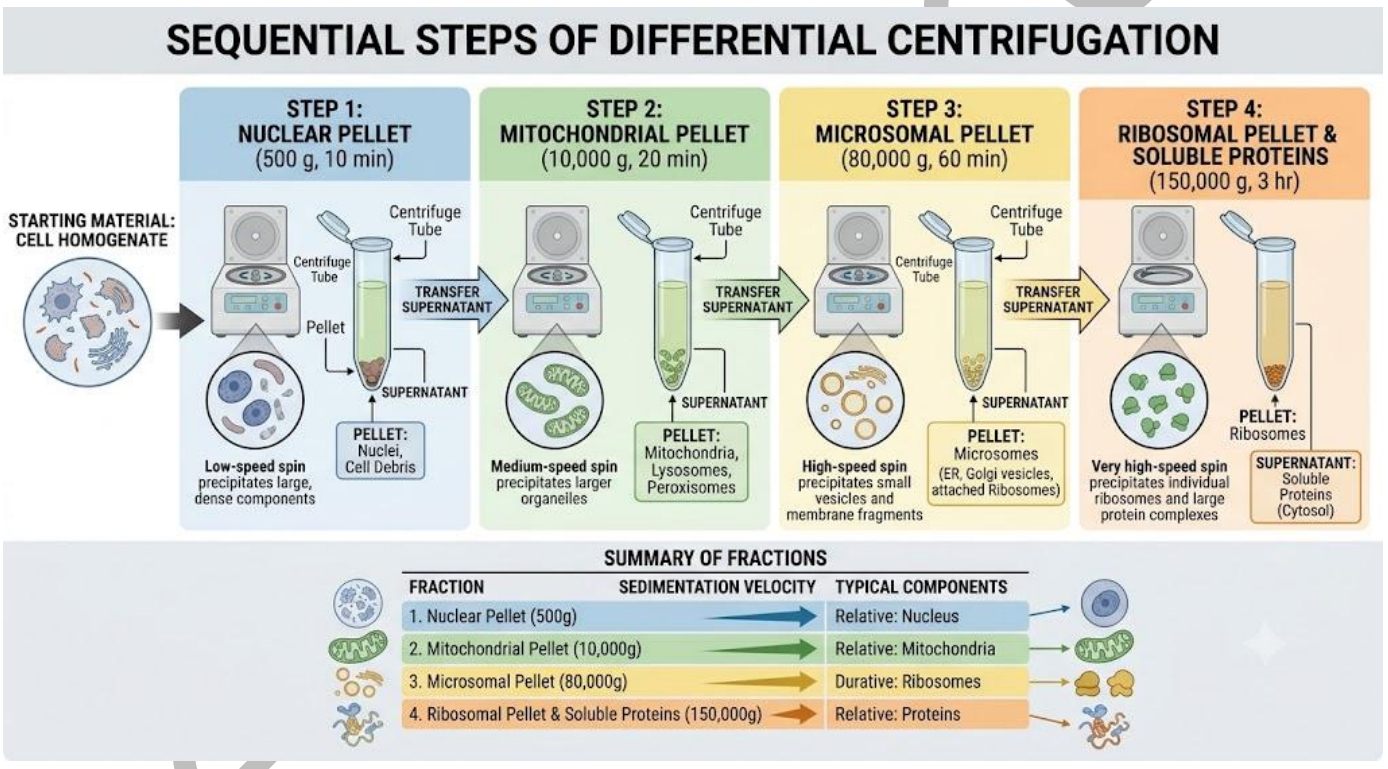

Figure 2.3: Diagram of differential centrifugation showing sequential fractionation of cell organelles

2.4.2 Principles and procedure of electrophoresis

Electrophoresis separates charged molecules (proteins, nucleic acids) by their migration through a gel matrix under the influence of an electric field. Negatively charged molecules migrate toward the positive electrode (anode); positively charged molecules migrate toward the negative electrode (cathode). The rate of migration depends on the size, shape, and net charge of the

molecule and on the pore size of the gel matrix. Smaller molecules migrate faster through the gel than larger ones.

SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis): the detergent SDS coats all proteins with a uniform negative charge proportional to their size, eliminating charge differences; separation is then purely by size (molecular weight). Procedure: denature proteins with SDS and heat; load into wells in a polyacrylamide gel; apply a voltage; run for the required time; stain with Coomassie blue or silver stain; compare band positions with a molecular weight ladder (standard). Agarose gel electrophoresis separates DNA and RNA fragments by size; bands are visualised under UV light with ethidium bromide or SYBR Green stain.

Fill in the blank: In SDS-PAGE, the detergent SDS gives all proteins a uniform _____ charge proportional to their size, so separation is based entirely on molecular weight rather than the protein's natural charge.

2.4.3 Applications of centrifugation and electrophoresis

Applications of centrifugation: isolation of cell organelles for biochemical studies (differential centrifugation); separation of blood into plasma and cells (clinical centrifugation); purification of viruses and plasmids (ultracentrifugation); separation of cream from milk (industrial centrifugation); and urine analysis in clinical laboratories (low-speed centrifugation to sediment cells and casts). Centrifugation is a universal sample preparation step before most analytical procedures.

Applications of electrophoresis: protein profiling and identification by SDS-PAGE; DNA fingerprinting and forensic analysis (agarose gel electrophoresis of PCR products); diagnosis of haemoglobin disorders such as sickle cell disease (haemoglobin electrophoresis); Western blotting (proteins separated by SDS-PAGE then transferred to a membrane and detected with antibodies); Southern blotting (DNA fragments detected with labelled probes after gel electrophoresis). Electrophoresis is indispensable in molecular biology, forensics, clinical diagnostics, and food science.

Fill in the blank: Haemoglobin electrophoresis is used to diagnose _____ cell disease by separating haemoglobin A (normal) from haemoglobin S (sickle); the two forms migrate to different positions in the gel because they differ in net charge.

Pilot questions 2.4

6. Explain the principle of centrifugation and distinguish between low-speed and ultracentrifugation.
7. Describe the steps of differential centrifugation and name the organelles isolated at each step.
8. Explain the principle of SDS-PAGE and state why SDS is used.
9. State three applications of gel electrophoresis in biology.
10. Distinguish between differential centrifugation and density-gradient centrifugation.

Series Summary

This Series covered the major analytical techniques in biological laboratory work. Spectrophotometry applies Beer-Lambert Law to determine concentration from absorbance; a colorimeter is simpler and cheaper; flame photometry measures metal ion concentrations; polarimetry quantifies optically active substances; refractometry determines dissolved solid concentration from the refractive index. Chromatography separates mixtures by differential migration; Rf values identify compounds; PC and TLC use paper and silica gel; column chromatography is preparative; GC separates volatile compounds using a carrier gas.

Melting point identifies pure solids and reveals impurities through broadened melting ranges. The four colligative properties (vapour pressure lowering, boiling point elevation, freezing point depression, osmotic pressure) depend on solute particle number; osmotic pressure controls water movement across cell membranes. Centrifugation separates cell fractions by size and density; differential centrifugation uses sequential speeds; electrophoresis separates charged molecules in a gel by size; SDS-PAGE is used for proteins; agarose gel for DNA. These techniques collectively address SLOs 2.1 to 2.7.

Glossary of Terms

- **Absorbance:** the logarithm of the ratio of incident to transmitted light intensity; directly proportional to concentration by Beer-Lambert Law.
- **Beer-Lambert Law:** the principle that absorbance of a solution is directly proportional to the concentration of the absorbing substance and the optical path length: A = ecl.
- **Calibration curve:** a graph of absorbance plotted against known concentrations of a standard solution, used to determine the concentration of unknown samples.
- **Centrifugation:** a technique that separates particles or molecules in a liquid by spinning at high speed to generate centrifugal force; denser particles sediment faster.
- **Chromatography:** a separation technique based on differential migration of mixture components through a stationary phase carried by a mobile phase.
- **Colorimetry:** measurement of the absorbance of visible light by a coloured solution using coloured filters; used to determine concentration of coloured substances.
- **Colligative properties:** solution properties that depend on the number of solute particles per unit volume, not on their chemical identity; include vapour pressure lowering, boiling point elevation, freezing point depression, and osmotic pressure.
- **Differential centrifugation:** sequential centrifugation at increasing speeds to pellet different cell fractions: nuclei (600 g), mitochondria (10,000 g), ribosomes (100,000 g).
- **Electrophoresis:** separation of charged molecules (proteins, nucleic acids) through a gel matrix under an electric field; smaller molecules migrate faster.
- **Gas chromatography (GC):** chromatographic technique using an inert carrier gas as mobile phase and a heated column with a liquid stationary phase; separates volatile compounds.
- **Melting point:** the temperature at which a pure solid substance changes to liquid at atmospheric pressure; characteristic of each pure compound; lowered and broadened by impurities.
- **Mobile phase:** in chromatography, the liquid or gas that flows through the stationary phase, carrying the mixture components with it.
- **Monochromator:** a device (prism or diffraction grating) in a spectrophotometer that selects a specific narrow wavelength of light from a broad-spectrum light source.
- **Osmotic pressure:** the pressure required to prevent osmosis across a semipermeable membrane; the most biologically important colligative property; governs water movement into and out of cells.

- **Polarimetry:** measurement of the angle by which an optically active substance rotates plane-polarised light; used to determine concentration and purity of chiral compounds such as sugars and amino acids.
- **Refractive index:** the ratio of the speed of light in a vacuum to its speed in a substance; increases with solute concentration; measured by a refractometer to determine dissolved solid content.
- **Rf value:** retardation factor; the ratio of the distance moved by a spot to the distance moved by the solvent front in chromatography; used to identify compounds.
- **SDS-PAGE:** sodium dodecyl sulphate polyacrylamide gel electrophoresis; SDS denatures proteins and gives them a uniform negative charge proportional to size, enabling separation by molecular weight only.
- **Spectrophotometry:** measurement of the absorbance of light at a specific wavelength by a solution; used with Beer-Lambert Law to determine solute concentration.
- **Stationary phase:** in chromatography, the solid or liquid phase held in place through which the mobile phase moves; components interact with it to varying degrees, causing separation.
- **Ultracentrifugation:** centrifugation above 100,000 g; used to sediment ribosomes, viruses, large macromolecules, and in density-gradient separations.

Concept Check Questions [Series 2]

Objective Questions

6. Beer-Lambert Law states that absorbance is directly proportional to the _____ of the absorbing substance and the path length. (Linked to SLO 2.1)
7. The Rf value is calculated by dividing the distance moved by the spot by the distance moved by the _____ front. (Linked to SLO 2.4)
8. Impurities _____ the melting point of a pure solid and broaden its melting range. (Linked to SLO 2.5)
9. In SDS-PAGE, SDS gives all proteins a uniform _____ charge proportional to their size, so separation is by molecular weight alone. (Linked to SLO 2.7)
10. In differential centrifugation, nuclei sediment first at _____ g; ribosomes require ultracentrifugation at approximately 100,000 g. (Linked to SLO 2.6)

Short-Answer Questions

11. State Beer-Lambert Law and name four components of a spectrophotometer. (Linked to SLO 2.1)
12. Distinguish between paper chromatography and thin-layer chromatography. (Linked to SLO 2.3)
13. State the four colligative properties of solutions and explain the biological importance of osmotic pressure. (Linked to SLO 2.5)
14. Describe the steps of differential centrifugation and name the fraction isolated at each step. (Linked to SLO 2.6)
15. State three applications of gel electrophoresis in biology or medicine. (Linked to SLO 2.7)

Long-Answer Questions

14. Describe the principles of spectrophotometry, colorimetry, polarimetry, and refractometry; state one biological application of each. (Linked to SLOs 2.1, 2.2)
15. Describe the principle of chromatography; compare paper, TLC, column, and gas chromatography; explain the Rf value and how it is used. (Linked to SLOs 2.3, 2.4)
16. Describe the four colligative properties of solutions and their biological applications; explain melting point determination; describe centrifugation and electrophoresis with their applications. (Linked to SLOs 2.5, 2.6, 2.7)

Answers to Concept Check Questions [Series 2]

Objective Questions

6. Concentration.
7. Solvent.
8. Lower.
9. Negative.
10. 600 g.

Short-Answer Questions

11. Beer-Lambert Law: A = ecl; absorbance is proportional to concentration and path length. Components: light source; monochromator (selects wavelength); sample cuvette; detector (measures transmitted light); readout device.
12. PC uses chromatography paper (cellulose) as stationary phase; separation is slower and spots are less sharp; suitable for amino acids, sugars. TLC uses silica gel or alumina on a plate; faster, sharper spots, better reproducibility; colourless spots detected under UV or with locating agents.
13. Four colligative properties: vapour pressure lowering; boiling point elevation; freezing point depression; osmotic pressure. Osmotic pressure governs water movement across cell membranes by osmosis; it determines turgor in plant cells, crenation or haemolysis of red blood cells, and is the basis for designing isotonic intravenous fluids.
14. Step 1: homogenise tissue in buffer; centrifuge at 600 g; pellet contains nuclei and cell debris. Step 2: centrifuge supernatant at 10,000 g; pellet contains mitochondria and chloroplasts. Step 3: centrifuge at 100,000 g; pellet contains ribosomes and microsomes; supernatant is the soluble cytoplasm fraction.
15. Protein profiling and identification by SDS-PAGE; DNA fingerprinting and forensic analysis by agarose gel electrophoresis of PCR products; diagnosis of haemoglobin disorders (sickle cell disease) by haemoglobin electrophoresis.

Long-Answer Questions

14. Spectrophotometry: measures absorbance of light at a specific wavelength; Beer-Lambert Law A = ecl; components include light source, monochromator, cuvette, detector; used to determine protein and nucleic acid concentrations. Colorimetry: uses coloured filters; simpler and cheaper; used for glucose, haemoglobin, and chlorophyll measurement; blank corrects for background. Polarimetry: measures rotation of plane-polarised light by chiral molecules; angle of rotation proportional to concentration; used to measure sucrose in the sugar industry and for drug purity testing. Refractometry: measures refractive index of solutions (increases with solute concentration); used in the food industry to assess sugar content of fruit juice and honey, and clinically to estimate urine specific gravity.
15. Chromatography principle: mixture components separate by differential migration through a stationary phase driven by a mobile phase; components with greater affinity for the stationary phase move more slowly. PC: paper (cellulose) stationary phase; aqueous mobile phase in solvent

mixture; spots near baseline indicate low affinity; used for amino acids and sugars. TLC: silica gel on a plate; faster; colourless spots detected by UV or locating agents; used for lipids, drugs, plant pigments. Column: glass column packed with silica or resin; preparative scale; components elute from the bottom; used to isolate proteins. GC: inert carrier gas mobile phase; heated column; vaporised samples; separates volatile fatty acids, steroids, pollutants. Rf = distance moved by spot / distance moved by solvent front; Rf values identify compounds by comparison with standards; range 0 to 1.0.

16. Colligative properties: (1) vapour pressure lowering (solute reduces solvent vapour pressure; basis of osmosis); (2) boiling point elevation (used in autoclaving for sterilisation); (3) freezing point depression (cryoprotection; osmometry for clinical osmolality measurement); (4) osmotic pressure (governs water movement across membranes; determines turgor in plants; basis of intravenous fluid design). Melting point: characteristic temperature at which a pure solid melts; impurities lower and broaden the range; used to identify and assess purity of compounds. Centrifugation: centrifugal force sediments particles by size and density; differential centrifugation isolates nuclei (600 g), mitochondria (10,000 g), ribosomes (100,000 g); density-gradient gives higher resolution. Electrophoresis: charged molecules migrate through gel under electric field; smaller migrate faster; SDS-PAGE separates proteins by molecular weight (SDS gives uniform negative charge); agarose gel separates DNA for fingerprinting and diagnosis; applications include sickle cell diagnosis, Western blotting, forensic DNA profiling.

Answers to Pilot Questions [Series 2]

Part 2.1

6. Beer-Lambert Law: A = ecl; absorbance is proportional to concentration (c) and path length (l). Components: light source (tungsten or deuterium lamp); monochromator (prism or grating selects specific wavelength); sample cuvette (holds the solution); detector (photodiode measures transmitted light); readout (gives absorbance or transmittance value).
7. A spectrophotometer uses a monochromator to select a precise single wavelength; more sensitive and accurate; can work in UV and visible ranges; expensive. A colorimeter uses coloured glass filters to select a broad waveband; cheaper and simpler; limited to visible range; suitable for routine work but less precise.
8. A calibration curve is a graph of absorbance (y-axis) against known concentrations (x-axis) of a standard solution. To use it: measure the absorbance of an unknown sample at the same

wavelength; read off the corresponding concentration from the graph by locating the absorbance on the y-axis and reading the concentration from the x-axis.

9. Polarimetry: (1) determining sucrose concentration in the sugar industry; (2) testing purity of chiral drug molecules. Refractometry: (1) measuring salinity of water samples in ecology and aquaculture; (2) estimating sugar content of fruit juice and honey in the food industry.
10. Flame photometry: a sample solution is aspirated into a flame; metal ions in the sample emit light of characteristic wavelengths when excited; the intensity of light emitted is proportional to the concentration of the metal ion. Biological application: measuring sodium and potassium concentrations in blood serum and urine in clinical chemistry.

Part 2.2

6. Principle: mixture components separate because they migrate at different rates through a stationary phase when carried by a mobile phase; components with greater affinity for the stationary phase move more slowly. Stationary phase: the solid or liquid held in place (e.g. paper, silica gel, packed resin). Mobile phase: the liquid or gas that moves through the stationary phase carrying the mixture (e.g. organic solvent, inert gas).
7. Spot 1: Rf = 4.2 / 7.0 = 0.60. Spot 2: Rf = 2.6 / 7.0 = 0.37.
8. PC: stationary phase is chromatography paper (cellulose holding water); slower development; spots may be less distinct; no special equipment needed. TLC: stationary phase is silica gel or alumina on a rigid plate; faster; sharper spots; better reproducibility; can detect colourless compounds under UV or with sprayed locating agents (e.g. ninhydrin, iodine vapour).
9. Column: liquid mobile phase; packed solid stationary phase; room temperature; preparative (isolates large quantities). GC: gaseous mobile phase (carrier gas); liquid stationary phase in a heated column; only volatile samples; analytical (gives peaks on a chromatogram rather than visible spots).
10. Column chromatography: (1) isolating a specific enzyme from a cell extract using ion-exchange resin; (2) purifying a natural product compound from a plant extract. Gas chromatography: (1) analysis of fatty acid composition of lipid extracts from biological tissues; (2) detecting and quantifying environmental pollutants (e.g. pesticides) in water and soil samples.

Part 2.3

6. A pure compound melts sharply over a narrow range (0.5 to 1 degree Celsius). Impurities lower the melting point and broaden the range (the sample begins to soften earlier and takes longer to become fully liquid). By comparing the observed melting range with the literature value for the pure compound and noting the range width, the degree of purity can be assessed; a wide, depressed range indicates impurities.
7. Four colligative properties: vapour pressure lowering; boiling point elevation; freezing point depression; osmotic pressure. In common: all depend on the number of solute particles per unit volume of solvent (molal concentration), not on the chemical identity of the solute; electrolytes produce greater effects than non-electrolytes at the same molar concentration because they dissociate into more particles.
8. NaCl is an electrolyte that dissociates into two ions (Na+ and Cl-) in solution, producing 2 mol of particles per mol of NaCl. Glucose is a non-electrolyte and remains as 1 mol of particles per mol. Colligative effects depend on particle number; 1 mol/L NaCl produces approximately twice the freezing point depression of 1 mol/L glucose at the same molar concentration.
9. Intravenous fluids must be isotonic (matching the osmotic pressure of blood plasma, approximately 280 to 300 mOsm/kg) to prevent osmotic damage to red blood cells. A hypotonic IV fluid would cause cells to swell and lyse (haemolysis) by osmosis; a hypertonic fluid would cause cells to shrink (crenation). Normal saline (0.9% NaCl) and 5% glucose are designed to be isotonic for safe intravenous use.
10. Cryoprotection of biological specimens for storage: cryoprotectant solutions (glycerol, DMSO) lower the freezing point and prevent ice crystal formation in cells and tissues during freezing. Clinical osmometry: freezing point depression of blood plasma or urine measures total osmolality, used to diagnose dehydration, diabetes insipidus, and electrolyte disorders.

Part 2.4

6. Centrifugation principle: spinning a sample generates centrifugal force (RCF, in units of g); denser or larger particles sediment to the bottom of the tube faster. Low-speed (up to 5,000 g): sediments intact cells, cell debris, and precipitates; used in clinical labs for blood and urine. Ultracentrifugation (above 100,000 g): sediments ribosomes, viruses, and large macromolecules; requires a specialised refrigerated ultracentrifuge.

7. Step 1: homogenise tissue in buffer; centrifuge at 600 g for 10 min; pellet = nuclei and cell debris. Step 2: centrifuge supernatant at 10,000 g for 20 min; pellet = mitochondria and chloroplasts. Step 3: centrifuge supernatant at 100,000 g for 60 min; pellet = ribosomes and microsomes (endoplasmic reticulum fragments). Remaining supernatant = soluble cytoplasmic proteins and metabolites.
8. Principle: proteins (denatured with SDS) migrate through polyacrylamide gel under an electric field; smaller proteins move faster. SDS is used because it coats all proteins with a uniform negative charge proportional to their mass, eliminating natural charge and shape differences so that migration depends only on molecular weight. Molecular weight is estimated by comparing band position with a protein ladder of known sizes.
9. Protein profiling and molecular weight determination (SDS-PAGE); DNA fingerprinting and forensic analysis (agarose gel, PCR products); diagnosis of sickle cell disease by haemoglobin electrophoresis; Western blotting for detecting specific proteins with antibodies.
10. Differential centrifugation: uses successive increases in speed to pellet different cell fractions by size and density; simple but separations are not fully pure (fractions may overlap). Density-gradient centrifugation: sample is centrifuged through a continuous or step gradient (sucrose or CsCl); particles migrate to the position in the gradient matching their own density (isopycnic banding); gives higher resolution and purer fractions than differential centrifugation; used to purify viruses and DNA.

References and Attributions [Series 2]

Textual References (Books and External Sources)

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Figures, Plates, Tables, and Boxes

- Figure 2.1: Spectrophotometer diagram and Beer-Lambert calibration curve Attribution: AI generated. Suggested OER search string: "spectrophotometer diagram Beer-Lambert calibration curve absorbance concentration OER".
- Figure 2.2: Paper chromatography setup and Rf value calculation diagram Attribution: AI generated. Suggested OER search string: "paper chromatography diagram setup Rf value calculation solvent front OER".
- Figure 2.3: Differential centrifugation diagram showing sequential cell fractionation Attribution: AI generated. Suggested OER search string: "differential centrifugation diagram cell fractionation nuclei mitochondria ribosomes OER".
- Box 2.1: Summary of colligative properties and their biological relevance Attribution: AI generated. Suggested OER search string: "colligative properties solutions boiling point elevation freezing point depression osmotic pressure table OER".

Course code: BIO 204

Course title: Biological Techniques

Course credit load: 2 Units

Series 3: Herbarium and Museum Techniques

- **Part 3.1: Collection and Pressing of Plant Specimens**
  - Sub Part 3.1.1: Field collection of plant specimens
  - Sub Part 3.1.2: Pressing and drying of specimens
  - Sub Part 3.1.3: Mounting and labelling herbarium sheets
- **Part 3.2: Herbarium Organisation and Management**
  - Sub Part 3.2.1: Storage, preservation, and pest control
  - Sub Part 3.2.2: Classification and curation of herbarium collections
  - Sub Part 3.2.3: Uses and importance of herbaria
- **Part 3.3: Collection and Preservation of Animal Specimens**
  - Sub Part 3.3.1: Methods of collecting animal specimens
  - Sub Part 3.3.2: Killing, fixing, and preserving animal specimens
  - Sub Part 3.3.3: Mounting and storing animal specimens
- **Part 3.4: Museum Organisation and Specimen Documentation**
  - Sub Part 3.4.1: Museum storage systems and curation
  - Sub Part 3.4.2: Labelling, cataloguing, and documentation
  - Sub Part 3.4.3: Uses and importance of biological museums

Introduction

Herbaria and biological museums are permanent repositories of plant and animal specimens collected, preserved, and documented for scientific study, education, and conservation. A herbarium holds dried, pressed plant specimens mounted on sheets; a biological museum holds preserved animal specimens in fluid, as dry mounts, or as skeletal preparations. Both require systematic collection, correct preservation, and accurate documentation to retain their scientific value over decades and centuries.

This Series covers field collection techniques for plants and animals, pressing and drying of plant material, mounting on herbarium sheets, fluid preservation and dry mounting of animal specimens, curation and cataloguing practices, and the scientific and conservation importance of both institutions. These skills build directly on the observational and drawing techniques covered in Series 1.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- SLO 3.1 describe the correct procedure for collecting plant specimens in the field (Linked to Part 3.1),
- SLO 3.2 explain the stages of pressing, drying, mounting, and labelling herbarium specimens (Linked to Part 3.1),
- SLO 3.3 describe the storage, pest control, classification, and curation of a herbarium collection (Linked to Part 3.2),
- SLO 3.4 state the uses and importance of herbaria in taxonomy, ecology, and conservation (Linked to Part 3.2),
- SLO 3.5 describe the methods of collecting, killing, fixing, and preserving animal specimens (Linked to Part 3.3),

- SLO 3.6 explain the mounting and storage of animal specimens in a biological museum (Linked to Part 3.3), and
- SLO 3.7 describe cataloguing and documentation practices and state the scientific importance of biological museums (Linked to Part 3.4).

3.1 Collection and Pressing of Plant Specimens

3.1.1 Field collection of plant specimens

A good herbarium specimen must represent the plant fully. Collect branches bearing leaves, flowers, and fruit or seeds where possible, since these are essential for identification. Use sharp secateurs or a knife to cut a branch 30 to 45 cm long. For small herbs, collect the whole plant including roots. Place specimens immediately into a polythene bag or a field press (a folded newspaper inside a plant press) to prevent wilting. Collect duplicate specimens (at least two) of each plant: one for the herbarium and one as a spare or for exchange.

Field notes are as important as the specimen itself. Record in a field notebook: collection number (a unique sequential number assigned to each collection); date; locality (GPS coordinates or a precise description); habitat (forest, savanna, riverbank); plant habit (tree, shrub, herb, climber); height; bark colour and texture; flower colour and scent (characters that are lost on drying); and the collector's name. The collection number links the specimen in the press to its field notes and later to the herbarium label. Ethical collection requires not over-collecting from rare or protected species.

Fill in the blank: Each plant collection is given a unique _____ number in the field notebook; this number links the specimen to its field notes recording date, locality, habitat, and morphological characters that may be lost on drying.

3.1.2 Pressing and drying of specimens

Pressing removes moisture and flattens the specimen for permanent storage. Equipment: a plant press consisting of two wooden lattice frames (driers) with corrugated aluminium or cardboard

ventilators, absorbent blotting paper or newspaper, and straps or ropes to apply pressure. Procedure: open the specimen carefully on a folded sheet of newspaper; arrange leaves so that both upper and lower surfaces are visible (fold some leaves to show the lower surface); spread flowers open if possible; place the newspaper with the specimen between two sheets of blotter paper, then between ventilators; stack multiple specimens and tighten the press firmly.

Drying: change the blotting paper daily for the first three to five days to remove moisture rapidly; slow drying causes browning and mould. In humid climates, place the press in a drying oven (50 to 60 degrees Celsius) or over a gentle heat source. Most specimens dry completely within one to two weeks. Succulent plants (e.g. cacti) require special treatment: briefly dip in boiling water or scald with steam to kill the tissue before pressing, or freeze before pressing, to prevent continued growth and rotting in the press.

Fill in the blank: Blotting paper in a plant press must be changed daily for the first three to five days to remove _____ rapidly and prevent mould and browning of the specimen during drying.

3.1.3 Mounting and labelling herbarium sheets

A herbarium sheet is a standard-sized (42 x 29 cm or A3) stiff white cardboard. The dried specimen is attached using narrow strips of gummed linen tape or archival glue applied across stems, petioles, and the midrib of larger leaves; do not cover flowers or fruits. The standard herbarium label (approximately 10 x 7 cm) is glued to the lower right corner of the sheet and must contain: family; genus and species (with author citation); collection number; collector's name; date; country and locality; habitat; altitude; and any field notes on habit, flower colour, or local name.

A fragment packet (a folded paper envelope) is glued to the lower left corner of the sheet to hold detached leaves, fruits, seeds, or flowers that fall off during handling. A determination slip is added when a taxonomist identifies or confirms the name; it records the identifier's name and date. Annotation labels record subsequent name changes or additional information. The sheet is now a permanent scientific record: all labels, handwriting, and additional material become part of the specimen's history and must never be removed.

Fill in the blank: The herbarium label is glued to the lower _____ corner of the sheet and must include the collector's name, collection number, date, locality, habitat, and the scientific name of the plant.

Figure 3.1: A correctly mounted and labelled herbarium sheet

Pilot questions 3.1

11. State four pieces of information that must be recorded in field notes when collecting a plant specimen.
12. Describe the procedure for pressing a plant specimen.
13. Explain why blotting paper must be changed daily during the first few days of pressing.
14. State six items of information that must appear on a herbarium label.
15. Explain the purpose of the fragment packet on a herbarium sheet.

3.2 Herbarium Organisation and Management

3.2.1 Storage, preservation, and pest control

Mounted herbarium sheets are stored vertically in herbarium folders (covers of stiff card) stacked in steel or wooden herbarium cupboards (pigeon-hole cabinets). Each folder holds the sheets of one species or genus. Folders are arranged systematically within the cupboard (by family, genus, and species in alphabetical or phylogenetic order). Cupboards must be kept closed to exclude light, dust, and insects. The herbarium room should be cool, dry, and well-ventilated; high humidity encourages mould, and high temperature accelerates deterioration of specimens.

Pest control is critical: insect pests (particularly dermestid beetles, booklice, and silverfish) destroy dried plant material. Methods: fumigation using naphthalene or paradichlorobenzene repellent blocks placed in cupboards; periodic treatment with ethyl acetate or carbon dioxide in sealed chambers; freezing infested material at -20 degrees Celsius for 72 hours to kill insects without chemical damage to specimens. New incoming specimens must always be frozen before shelving to prevent introducing pests. Toxic older preservatives (mercuric chloride, arsenic) must be handled with gloves as they remain on historical specimens.

Fill in the blank: New plant specimens arriving at a herbarium must be _____ at -20 degrees Celsius for 72 hours before being shelved, to prevent introducing insect pests such as dermestid beetles into the existing collection.

3.2.2 Classification and curation of herbarium collections

Herbarium collections are arranged taxonomically: sheets are grouped by species within genus, genera within family, and families arranged in a recognised systematic sequence (e.g. the Cronquist or APG system for flowering plants). Within a species, sheets may be further arranged geographically or chronologically. A type specimen (holotype) is the single specimen on which the original description and name of a species was based; it has special legal and scientific status and is usually kept in a separate type cabinet within the herbarium.

Curation involves ongoing maintenance: re-annotating specimens when names change; repairing damaged sheets; adding determination slips; digitising specimen data into a database; and producing loans to other herbaria for taxonomic study. A curator or herbarium technician is responsible for the physical condition and organisation of the collection. Accession registers record every specimen added to the collection with a unique accession number, enabling retrieval and tracking of loans.

Fill in the blank: The single specimen on which the original description and name of a species is formally based is called the _____ specimen (holotype); it has special legal and scientific status and is kept separately in a type cabinet.

3.2.3 Uses and importance of herbaria

Herbaria serve multiple scientific functions: taxonomic reference (comparing new collections with existing named specimens to identify plants); type specimen repository (preserving the nomenclatural standard for each species name); floristic surveys (determining which plant species occur in a region, essential for biodiversity assessments); ecological and climate change research (historical specimens document past distributions and phenology, enabling comparison with current conditions); and ethnobotanical records (preserving documentation of medicinal and useful plants).

In Nigeria, the main herbaria are at the Federal Department of Forestry (Lagos), University of Ibadan, and Ahmadu Bello University (Zaria). They hold collections of Nigerian flora essential for agricultural pest identification, medicinal plant research, and forest conservation. Digital herbaria (online databases with scanned specimen images) now allow global access to collection data; platforms such as GBIF (Global Biodiversity Information Facility) aggregate specimen records from herbaria worldwide, making them available for conservation planning and ecological modelling.

Fill in the blank: Historical herbarium specimens enable climate change researchers to compare past plant _____ (the timing of flowering and fruiting) with current observations, providing evidence of shifts in plant responses to temperature and rainfall over decades.

Figure 3.2: Interior of a herbarium showing cupboards, folders, and specimen arrangement

Pilot questions 3.2

11. Describe how herbarium sheets are stored in a herbarium cupboard.
12. State three methods used to control insect pests in herbaria.
13. Explain what a type specimen (holotype) is and why it is significant.
14. State four uses of herbaria in biological research.
15. Name two Nigerian herbaria and state the significance of digital herbarium databases such as GBIF.

3.3 Collection and Preservation of Animal Specimens

3.3.1 Methods of collecting animal specimens

Collection methods vary by animal group. Insects: sweep nets (for flying and foliage insects); pitfall traps (buried containers for ground-dwelling beetles and ants); light traps (ultraviolet light attracts nocturnal moths and beetles); Malaise traps (tent-like structures that intercept flying insects); pooters (aspirators that suck small insects into a collection vial). Aquatic invertebrates:

kick sampling with a pond net; Surber samplers for stream benthos; plankton tow nets for water-column organisms.

Vertebrates: fish are collected using seine nets, gill nets, or anaesthetic (e.g. clove oil, MS-222) in enclosed water bodies; amphibians by hand at night or drift fences with pitfall arrays; reptiles by hand or noose poles; birds by mist nets or shooting (museum specimens); mammals by live traps (Sherman traps for small rodents), pitfall arrays, or, historically, shooting. All vertebrate collection must comply with institutional animal ethics guidelines and relevant national wildlife laws; permits are required in Nigeria under the Endangered Species Act.

Fill in the blank: A _____ trap is a tent-like structure erected across a flight path that intercepts flying insects; they fall or walk upward into a collecting jar at the apex and are used to sample insect diversity over extended periods.

3.3.2 Killing, fixing, and preserving animal specimens

Killing: insects are killed in a killing jar containing a few drops of ethyl acetate on a plaster of Paris base; the vapour anaesthetises and kills the insect without damaging external features. Cyanide killing jars were historically used but are now avoided due to extreme toxicity. Aquatic invertebrates and soft-bodied animals are killed by dropping into boiling water or 70% alcohol, or by relaxing in menthol crystals (for marine invertebrates) before fixation. Vertebrates are killed humanely per ethics guidelines (anaesthetic overdose; CO_2 chamber for small mammals).

Fixation: formalin (4% formaldehyde) is injected into the body cavity of fish, frogs, and reptiles and the specimen is submerged in formalin for 24 to 48 hours; this cross-links proteins and hardens the tissue, preventing autolysis and bacterial decay. After fixation, specimens are transferred to 70% ethanol for long-term storage (formalin damages DNA; ethanol is preferred when DNA extraction may be needed later). Invertebrate soft tissues are fixed in Bouin's fluid or glutaraldehyde for histological study.

Fill in the blank: After initial fixation in _____ for 24 to 48 hours, vertebrate museum specimens are transferred to 70% ethanol for long-term storage because formalin damages DNA, making ethanol the preferred preservative when molecular studies are anticipated.

3.3.3 Mounting and storing animal specimens

Insects are mounted on entomological pins through the right side of the thorax (for most insects) or through the right elytron for beetles. The pin is pushed through a pinning block to standardise the height of the specimen on the pin; wings are spread on a spreading board and held in position with paper strips until dry (24 to 72 hours for small insects; one to two weeks for large moths). Relaxing chambers (a closed container with damp sand or wet paper towel) are used to soften dried insects before mounting.

Fluid specimens (fish, amphibians, reptiles, invertebrates in alcohol) are stored in glass or plastic jars with tight-fitting lids; labels written in pencil or with waterproof Indian ink are placed inside the jar (never written on the outside, as external labels fade and detach). Skeletal preparations are made by maceration (soaking in water until soft tissue decomposes), dermestid beetle colonies (beetles consume soft tissue cleanly), or enzyme digestion; bones are then degreased, bleached lightly, and dried. Study skins of birds and mammals are prepared by skinning, stuffing with cotton wool, and sewing closed.

Fill in the blank: Insect specimens are mounted on entomological pins pushed through the right side of the _____ (the middle body region); the pin height is standardised using a pinning block before the label is added below the specimen.

Figure 3.3: Methods of mounting and storing animal specimens

Pilot questions 3.3

11. Name four methods used to collect insects in the field.
12. Explain the purpose of fixation in formalin and state why specimens are later transferred to ethanol.
13. Describe the correct procedure for pinning an insect specimen.
14. State two methods of preparing skeletal specimens for a museum collection.
15. Explain why labels for fluid specimens must be placed inside the jar rather than written on the outside.

3.4 Museum Organisation and Specimen Documentation

3.4.1 Museum storage systems and curation

A biological museum (natural history collection) organises specimens by taxonomic group in a series of dedicated storage areas: an entomology section (pinned insects in glass-topped drawers

inside wooden or metal cabinets); a herpetology and ichthyology section (fluid specimens in jars on open metal shelving); a mammalogy and ornithology section (study skins, skulls, and skeletal material in flat trays inside cabinets); and, where available, a frozen tissue collection (-80 degrees Celsius freezers holding tissue samples for DNA work).

Pest control in museums mirrors herbarium practice: naphthalene or paradichlorobenzene in insect drawers; regular monitoring for dermestid beetle activity; freezing of incoming dry material; maintaining low humidity (below 50%) to prevent mould on study skins and skeletal specimens. The curator must regularly inspect all collections, top up evaporating fluid in jars, replace degraded labels, and update the database. Environmental monitoring (temperature, relative humidity, light levels) is recorded continuously in modern museum facilities.

Fill in the blank: The relative humidity in a biological museum storage area should be kept below _____ percent to prevent mould growth on study skins and skeletal specimens; temperature and light levels are also monitored continuously.

3.4.2 Labelling, cataloguing, and documentation

Every specimen in a biological museum must carry a label bearing: a unique catalogue (accession) number; the scientific name (family, genus, species, and author); the collector's name and collection number; the date and precise locality of collection; the habitat and notes on the live animal (colour, behaviour, measurements); and any subsequent identifications or name changes. Labels for fluid specimens are written in waterproof Indian ink on rag paper (acid-free); paper labels in alcohol remain legible for decades. Pin labels for insects use small printed or typed slips below the specimen on the pin.

Catalogues and databases: each specimen is entered in a collection register (traditionally a bound ledger; now a relational database) with its accession number, taxon, locality, collector, date, and storage location. Digitisation programmes photograph specimens and geo-reference locality data, loading all information into publicly accessible databases (e.g. VertNet for vertebrate collections; iDigBio). Standard body measurements (total length, body length, tail length, mass) are recorded for vertebrate specimens; wing length and forewing pattern for insects; standard meristic counts for fish.

Fill in the blank: Labels for fluid specimens in biological museums are written in waterproof _____ ink on acid-free rag paper and placed inside the jar because external labels written on glass fade and detach over time.

3.4.3 Uses and importance of biological museums

Biological museums serve as: taxonomic reference collections (specimens are compared with described species to confirm identification; type specimens are the authoritative standard); biodiversity archives (documenting species richness and geographic distributions over time); sources of tissue for molecular systematics, population genetics, and ancient DNA studies; records of historical baselines for ecological change (morphometric shifts, parasite loads, disease prevalence in historical specimens compared with current samples); and educational resources for university teaching and public engagement.

In Nigeria, the Natural History Museum at the University of Ibadan and collections at Ahmadu Bello University hold regionally significant vertebrate and invertebrate series. These collections document Nigerian biodiversity before the large-scale habitat loss of the late 20th century, providing baseline data for conservation assessments. International exchange (loans between museums) allows systematic revision of taxa by specialist taxonomists who may be based anywhere in the world. Without these collections, much of our knowledge of African biodiversity would be irretrievably lost.

Fill in the blank: Biological museum specimens can provide tissue for _____ studies, enabling researchers to sequence DNA from historical specimens and compare it with modern populations to study evolution, population decline, and disease history over time.

Figure 3.4: Interior of a biological museum showing fluid and dry specimen storage and the catalogue workstation

Pilot questions 3.4

11. Describe the storage arrangements for insect, fluid, and study skin specimens in a biological museum.
12. State six items of information that must appear on the label of a museum specimen.
13. Explain the purpose of digitisation in museum curation and name one global database for vertebrate collections.
14. State four uses of biological museums in scientific research.
15. Explain why the biological museum collections at the University of Ibadan are particularly important for Nigerian conservation.

Series Summary

This Series covered the principles and practices of herbarium and museum techniques. Plant specimens are collected in the field with full notes, pressed in a plant press with daily blotter changes, dried, mounted on standard herbarium sheets with gummed tape, and labelled with collection data. Herbaria are organised taxonomically in pigeon-hole cupboards; pest control uses freezing and fumigation; type specimens are stored separately; curation involves re-annotation, database entry, and loans. Herbaria serve taxonomy, floristics, ecology, and conservation research.

Animal specimens are collected using group-specific methods (sweep nets, pitfall traps, seine nets, mist nets); killed humanely or in ethyl acetate jars; fixed in formalin then transferred to 70% ethanol; or dry-mounted as pinned insects, study skins, or skeletal preparations. Museum storage is taxonomically organised; labels use waterproof ink on acid-free paper placed inside fluid jars; databases record accession data. Biological museums underpin taxonomy, molecular systematics, biodiversity monitoring, and conservation. These skills collectively address SLOs 3.1 to 3.7.

Glossary of Terms

- **Accession number:** a unique sequential number assigned to each specimen entered into a museum or herbarium collection; used for retrieval, cataloguing, and loan tracking.
- **Annotation label:** a small label added to a herbarium sheet or museum specimen recording a subsequent name change, additional identification, or new information discovered after the original mounting.
- **Collection number:** a unique sequential number assigned to each plant or animal collection in the field; links the specimen to the collector's field notes and to the herbarium or museum label.
- **Curator:** the scientist or technician responsible for the organisation, maintenance, and scientific integrity of a herbarium or museum collection.
- **Determination slip:** a small label added to a herbarium sheet by a taxonomist confirming or revising the identification of the specimen, with the identifier's name and date.

- **Fixation:** the chemical treatment of biological tissue (using formalin or glutaraldehyde) to cross-link proteins, halt autolysis, and prevent bacterial decay; preserves tissue structure for long-term storage.
- **Fragment packet:** a small folded paper envelope glued to the lower left corner of a herbarium sheet to hold detached plant parts (leaves, seeds, fruits, flowers) that separate from the specimen during handling.
- **GBIF:** Global Biodiversity Information Facility; an international online database aggregating specimen records from herbaria and museums worldwide, enabling global access for conservation and ecological research.
- **Herbarium:** a systematically arranged collection of dried, pressed plant specimens mounted on herbarium sheets and stored in labelled folders in cupboards; used for taxonomic reference, floristic surveys, and biodiversity research.
- **Holotype:** the single designated specimen on which the original formal description and name of a species is based; the ultimate nomenclatural standard for that species name.
- **Killing jar:** a sealed glass container charged with ethyl acetate vapour used to kill insects rapidly without damaging their external features; used in entomological collecting.
- **Maceration:** a method of skeletal preparation in which a carcass is soaked in water until soft tissue decomposes and can be removed, leaving clean bones; used for medium to large vertebrates.
- **Malaise trap:** a tent-like collecting device erected in an insect flight path; insects flying into the trap are directed upward into a collecting jar; used to sample flying insect diversity over extended periods.
- **Phenology:** the study of the timing of periodic biological events such as flowering, fruiting, migration, and breeding; historical herbarium specimens document past phenological patterns.
- **Pinning block:** a tiered wooden or metal block with grooves at standard depths used to position insect specimens and their labels at uniform heights on the entomological pin.
- **Plant press:** a device for pressing and drying plant specimens consisting of two wooden lattice frames with ventilators, blotter paper, and straps; used to produce flat, dried herbarium specimens.
- **Relaxing chamber:** a sealed container containing damp material (sand, wet paper towel) used to rehydrate dried insect specimens to make them pliable enough for mounting without breakage.
- **Spreading board:** a grooved board on which pinned insects with wings (butterflies, moths, dragonflies) are positioned with wings spread and held by paper strips until dry.

- **Study skin:** a preserved bird or mammal skin stuffed with cotton wool into a standard posture and labelled; used as a museum reference specimen for morphological study and comparison.

Concept Check Questions [Series 3]

Objective Questions

11. Each plant collection is assigned a unique _____ number in the field notebook that links the specimen to its locality, date, and morphological notes. (Linked to SLO 3.1)
12. The standard herbarium label is glued to the lower _____ corner of the mounted sheet. (Linked to SLO 3.2)
13. The single specimen on which a species name is formally based is the _____ (holotype); it is kept in a separate type cabinet. (Linked to SLO 3.3)
14. After fixation in formalin, vertebrate specimens are transferred to 70% _____ for long-term storage because formalin degrades DNA. (Linked to SLO 3.5)
15. Labels for fluid specimens are placed _____ the jar and written in waterproof Indian ink on acid-free paper. (Linked to SLO 3.7)

Short-Answer Questions

16. State four items of information recorded in plant collection field notes. (Linked to SLO 3.1)
17. Describe the procedure for pressing and drying a plant specimen. (Linked to SLO 3.2)
18. State three methods of controlling insect pests in a herbarium or museum. (Linked to SLO 3.3)
19. Distinguish between fixation in formalin and long-term storage in ethanol for animal specimens. (Linked to SLO 3.5)
20. State four uses of biological museum collections in research. (Linked to SLO 3.7)

Long-Answer Questions

17. Describe the complete procedure for collecting, pressing, drying, mounting, and labelling a plant specimen for a herbarium, and state the scientific importance of herbaria. (Linked to SLOs 3.1, 3.2, 3.3, 3.4)
18. Describe the methods of collecting, killing, fixing, and preserving animal specimens; explain how insects and vertebrates are stored and mounted in a biological museum. (Linked to SLOs 3.5, 3.6)

19. Describe the labelling, cataloguing, and digitisation of biological museum specimens, and explain the scientific and conservation importance of museum collections with reference to Nigeria. (Linked to SLOs 3.6, 3.7)

Answers to Concept Check Questions [Series 3]

Objective Questions

11. Collection.
12. Right.
13. Type specimen.
14. Ethanol.
15. Inside.

Short-Answer Questions

16. Collection number; date; precise locality (GPS or description); habitat type; plant habit and height; flower colour and scent (lost on drying); collector's name.
17. Place specimen on newspaper inside the press; arrange leaves to show both surfaces; spread flowers; sandwich between blotter paper and ventilators; tighten press straps. Change blotter daily for 3 to 5 days. Dry at 50 to 60 degrees Celsius if humid. Specimens dry in 1 to 2 weeks.
18. Freezing new material at -20 degrees Celsius for 72 hours before shelving; placing naphthalene or paradichlorobenzene repellent blocks in cupboards or drawers; periodic fumigation with ethyl acetate or CO2 in sealed chambers.
19. Formalin (4% formaldehyde) is injected into the body cavity and hardens tissue by cross-linking proteins, preventing autolysis and decay; used for 24 to 48 hours only. Ethanol (70%) is used for long-term storage because it preserves tissue without degrading DNA; formalin cross-links DNA and makes it unsuitable for molecular studies.
20. Taxonomic reference (comparing specimens with described species); type specimen repository (nomenclatural standards); biodiversity archives (documenting species distributions over time); source of tissue for DNA and molecular systematics; historical baselines for ecological and climate change research.

Long-Answer Questions

17. Collection: cut a 30 to 45 cm branch bearing leaves, flowers, and fruit; record collection number, date, locality, habitat, habit, flower colour in field notebook; place in polythene bag or field press; collect duplicates. Pressing: arrange on newspaper in press; show both leaf surfaces; change blotter daily for 3 to 5 days; dry at 50 to 60 degrees Celsius; full drying in 1 to 2 weeks. Mounting: attach dried specimen to A3 herbarium sheet with gummed linen tape strips; place standard label (lower right corner) with family, name, collector, collection number, date, and locality; add fragment packet (lower left) for loose parts; add determination slip when identified. Importance: taxonomic reference; type specimen repository; floristic surveys; ecological and climate change research using historical specimens; ethnobotanical records; digital databases (GBIF) give global access for conservation.
18. Collection methods: insects (sweep net, pitfall trap, light trap, Malaise trap, pooter); fish (seine net, gill net, anaesthetic); amphibians and reptiles (hand, drift fences, noose poles); birds (mist nets); small mammals (Sherman live traps). Killing: insects in ethyl acetate killing jar; vertebrates by anaesthetic overdose or CO2. Fixation: inject 4% formalin; submerge for 24 to 48 hours; transfer to 70% ethanol for long-term storage (formalin degrades DNA). Insect mounting: pin through right thorax (or right elytron for beetles); use pinning block for standard height; spread wings on spreading board; dried in 24 hours to 2 weeks. Vertebrate storage: fluid specimens (fish, amphibians, reptiles) in glass jars of 70% ethanol with internal waterproof ink labels on acid-free paper. Bird and mammal study skins: skin, stuff with cotton wool in standard posture, sew, attach leg tag with data. Skeletal preparation: maceration in water, dermestid beetles, or enzyme digestion; degrease and dry bones.
19. Labelling: every specimen carries a unique accession number; label states scientific name, collector, collection number, date, locality, habitat, and live animal notes; fluid labels in waterproof Indian ink on acid-free rag paper placed inside the jar; insect pin labels are small typed slips below the specimen. Cataloguing: entries in collection register (bound ledger or database) include accession number, taxon, locality, collector, date, and storage location. Digitisation: specimens photographed; locality data geo-referenced; records uploaded to online databases (VertNet for vertebrates; iDigBio) for global access. Scientific and conservation importance: taxonomic reference; type specimens; biodiversity archives documenting historical distributions; tissue for DNA and molecular studies; ecological baselines for change detection. Nigerian importance: University of Ibadan Natural History Museum and ABU Zaria collections document pre-habitat-loss Nigerian biodiversity; support conservation assessments, agricultural

pest identification, and medicinal species research; international loans enable expert taxonomic revision of Nigerian taxa.

Answers to Pilot Questions [Series 3]

Part 3.1

11. Collection number; date of collection; precise locality (GPS coordinates or detailed description); habitat type (e.g. forest, savanna, riverbank); plant habit and height; flower colour and scent; bark colour and texture; collector's name.
12. Lay the specimen on a sheet of newspaper inside the press; arrange leaves so both upper and lower surfaces are visible; spread flowers open. Place newspaper between two sheets of blotter paper; add ventilators on either side; stack specimens and tighten straps firmly. Change blotter daily for 3 to 5 days. Dry in an oven at 50 to 60 degrees Celsius; specimens are fully dried in 1 to 2 weeks.
13. Blotter paper absorbs moisture released from the specimen; if not changed, saturated blotter cannot absorb further moisture, causing the specimen to remain damp, which promotes mould growth and discolouration (browning) of the plant tissue before it dries.
14. Family; scientific name (genus, species, and author); collection number; collector's name; date of collection; country and precise locality; habitat; altitude; notes on habit, flower colour, and local name.
15. The fragment packet (a folded paper envelope in the lower left corner) holds plant parts that detach from the specimen during handling (leaves, seeds, fruits, flowers); this ensures all material from the collection remains with the sheet and is not lost, preserving the completeness of the scientific record.

Part 3.2

11. Mounted sheets are placed vertically inside herbarium folders (stiff card covers) stacked in steel or wooden pigeon-hole cupboards; folders are labelled by genus and species; cupboards are labelled by family; the room is kept cool, dry, and well-ventilated with cupboard doors closed to exclude light, dust, and insects.
12. Freezing incoming material at -20 degrees Celsius for 72 hours before shelving; placing naphthalene or paradichlorobenzene repellent blocks inside cupboards; periodic fumigation of affected material with ethyl acetate or CO2 in a sealed chamber.

13. A holotype is the single designated specimen on which the formal description and name of a species is based; it is the ultimate nomenclatural standard. Its significance: if the identity of a species is disputed, the holotype is the final reference; no other specimen takes precedence. It is therefore kept separately in a type cabinet with special care and never loaned without extraordinary justification.
14. Taxonomic reference (comparing new collections with named specimens to confirm identity); type specimen repository; floristic surveys to determine regional species richness; ecological and climate change research using historical specimen data (distributions and phenology); ethnobotanical documentation of medicinal and useful plants.
15. Two Nigerian herbaria: Federal Department of Forestry Herbarium (Lagos) and University of Ibadan Herbarium. GBIF significance: it aggregates digitised specimen data from herbaria worldwide into a single searchable online platform, enabling researchers anywhere to access locality, taxonomy, and collection data for conservation planning, species distribution modelling, and biodiversity assessments without physically visiting each herbarium.

Part 3.3

11. Sweep net (for flying and foliage insects); pitfall trap (buried container for ground beetles and ants); light trap (UV light attracts nocturnal moths and beetles); Malaise trap (tent-like structure intercepting flying insects); pooter (aspirator sucking small insects into a vial).
12. Formalin (4% formaldehyde) is injected into the body cavity and the specimen submerged for 24 to 48 hours; it cross-links proteins, halts autolysis (self-digestion), and kills bacteria, hardening the tissue and preserving structure. Specimens are then transferred to 70% ethanol because formalin cross-links DNA and makes it unsuitable for molecular work; ethanol preserves tissue and keeps DNA extractable for future genetic studies.
13. Push the entomological pin through the right side of the thorax (or right elytron for beetles); use the pinning block to set the specimen at the standard height (approximately one-third from the pin head); spread wings on a spreading board if required; hold with paper strips until dry; add locality and identification labels below the specimen at standardised heights using the pinning block.
14. Maceration: soak the carcass in water until soft tissue decomposes; clean and dry bones. Dermestid beetle colony: beetles consume soft tissue cleanly from the bones; then degrease and dry. Both methods are followed by degreasing in acetone or ammonia and light bleaching if required.

15. External labels written on glass fade over time due to alcohol, abrasion, and light; they may also detach and become separated from the specimen, making the specimen unidentifiable. Labels placed inside the jar in 70% alcohol remain intact and legible for decades, ensuring the specimen and its data are never separated.

Part 3.4

11. Insects: pinned in glass-topped drawers inside locked wooden or metal cabinets; drawers contain naphthalene repellent. Fluid specimens (fish, amphibians, reptiles, invertebrates): stored in glass jars of 70% ethanol on open metal shelving, organised by taxon. Study skins (birds, small mammals): stored in flat trays inside cabinets, arranged taxonomically; skulls and skeletal material stored in separate labelled boxes.
12. Unique accession number; scientific name (family, genus, species, and author); collector's name and collection number; date of collection; precise locality; habitat and notes on the live animal (colour, measurements); any subsequent identifications or name changes.
13. Digitisation involves photographing specimens and geo-referencing their locality data; all information is entered into a searchable database and uploaded to publicly accessible online platforms. This allows researchers worldwide to access specimen data remotely without physical loans, accelerates taxonomic revisions, and enables large-scale biodiversity and distribution analyses. VertNet is a global online database for vertebrate museum collection records.
14. Taxonomic reference (confirming identifications against type and voucher specimens); type specimen repository (nomenclatural standards for species names); biodiversity archives (historical distributions and species richness); source of tissue for DNA, population genetics, and ancient DNA studies; ecological baselines for monitoring morphometric change, disease prevalence, and parasite loads over time.
15. The University of Ibadan Natural History Museum holds vertebrate and invertebrate collections assembled before the extensive deforestation and habitat conversion that occurred in Nigeria from the 1970s onwards; these specimens document species distributions, abundances, and morphology under original habitat conditions. They provide baseline data against which current surveys can be compared to measure biodiversity loss, support conservation status assessments, and inform policies for protected area management in Nigeria.

References and Attributions [Series 3]

Textual References (Books and External Sources)

- Bridson, D., & Forman, L. (1998). The herbarium handbook (3rd ed.). Royal Botanic Gardens, Kew.
- Hall, B. G. (2011). Phylogenetic trees made easy: A how-to manual (4th ed.). Sinauer Associates.
- Knapp, S., & Press, J. R. (1994). Applications of natural history collections. Systematics Association Special Volume.
- Martin, R. E. (1994). Collecting, preparing, and preserving insects, mites, and spiders. Agriculture Canada Publication.

Figures, Plates, Tables, and Boxes

- Figure 3.1: A correctly mounted and labelled herbarium sheet Attribution: AI generated. Suggested OER search string: "herbarium sheet mounted specimen label fragment packet correctly mounted OER photograph".
- Figure 3.2: Interior of a herbarium showing cupboards, folders, and specimen arrangement Attribution: AI generated. Suggested OER search string: "herbarium interior cupboards folders specimen arrangement curator OER photograph".
- Figure 3.3: Methods of mounting and storing animal specimens Attribution: AI generated. Suggested OER search string: "animal specimen mounting pinned insect fluid specimen bird study skin skeletal preparation museum OER photograph".
- Figure 3.4: Interior of a biological museum showing fluid and dry specimen storage and catalogue workstation Attribution: AI generated. Suggested OER search string: "natural history museum storage fluid specimens insect drawers database catalogue OER photograph".

Course code: BIO 204

Course title: Biological Techniques

Course credit load: 2 Units

Series 4: Experimental Design in Biology

- **Part 4.1: Scientific Method and Hypothesis Formulation**
  - Sub Part 4.1.1: The scientific method
  - Sub Part 4.1.2: Formulating hypotheses and research questions
  - Sub Part 4.1.3: Variables, controls, and replication
- **Part 4.2: Types of Experimental Design**
  - Sub Part 4.2.1: Completely randomised and randomised block designs
  - Sub Part 4.2.2: Factorial and Latin square designs
  - Sub Part 4.2.3: Observational studies and survey design
- **Part 4.3: Data Collection and Sampling Methods**
  - Sub Part 4.3.1: Types of data and measurement scales
  - Sub Part 4.3.2: Sampling strategies
  - Sub Part 4.3.3: Sources of error and bias
- **Part 4.4: Data Analysis and Interpretation**
  - Sub Part 4.4.1: Descriptive statistics
  - Sub Part 4.4.2: Inferential statistics and hypothesis testing
  - Sub Part 4.4.3: Graphical presentation and interpretation of results

Introduction

Experimental design is the systematic planning of an investigation to ensure that valid, reproducible conclusions can be drawn from the data collected. A poorly designed experiment

produces results that cannot be interpreted or trusted, regardless of how carefully the measurements are made. Understanding experimental design is therefore a core competency for any biologist working at the laboratory or field level.

This Series covers the steps of the scientific method, hypothesis formulation, identification of variables and controls, the major types of experimental design, sampling strategies, common sources of error, and the principles of data analysis and graphical presentation. These skills are directly applied in the research and report writing covered in Series 5.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- SLO 4.1 describe the steps of the scientific method and explain the role of observation, hypothesis, and experiment (Linked to Part 4.1),
- SLO 4.2 formulate a testable hypothesis and distinguish between null and alternative hypotheses (Linked to Part 4.1),
- SLO 4.3 identify independent, dependent, and controlled variables in an experiment and explain the purpose of controls and replication (Linked to Part 4.1),
- SLO 4.4 describe and compare the completely randomised, randomised block, factorial, and Latin square designs (Linked to Part 4.2),
- SLO 4.5 distinguish between types of data and measurement scales, and describe the major sampling strategies (Linked to Part 4.3),
- SLO 4.6 identify and minimise sources of error and bias in biological experiments (Linked to Part 4.3), and
- SLO 4.7 calculate descriptive statistics, apply basic inferential tests, and present results graphically (Linked to Part 4.4).

4.1 Scientific Method and Hypothesis Formulation

4.1.1 The scientific method

The scientific method is a systematic approach to acquiring reliable knowledge about the natural world. Its steps are: (1) observation (noticing a phenomenon or pattern that requires explanation); (2) question formulation (converting the observation into a specific, answerable question); (3) hypothesis formulation (proposing a testable explanation); (4) experimental design (planning how to test the hypothesis, including controls and replication); (5) data collection (carrying out the experiment and recording measurements); (6) data analysis (applying appropriate statistical methods); and (7) conclusion (accepting or rejecting the hypothesis based on evidence; communicating results).

Science is self-correcting: conclusions are provisional and subject to revision when new evidence emerges. Reproducibility is essential; other scientists must be able to repeat the experiment and obtain the same results. Peer review (evaluation of a study by independent experts before publication) is the mechanism by which the scientific community validates findings. A theory in science is not a guess; it is a well-supported, tested explanation that has survived repeated attempts at falsification (e.g. the theory of evolution by natural selection).

Fill in the blank: The step of the scientific method in which an independent expert evaluates a study before publication is called _____ review; it ensures the scientific community can scrutinise methods, data, and conclusions before they enter the literature.

4.1.2 Formulating hypotheses and research questions

A hypothesis is a specific, testable, falsifiable statement that proposes a relationship between variables. A good hypothesis: is based on prior observation or knowledge; makes a clear prediction; can be tested by experiment or observation; and can in principle be shown to be false

(falsifiability, as required by Popper's criterion of science). Example: "Increasing the concentration of fertiliser applied to maize seedlings will increase shoot height after four weeks." This is testable, falsifiable, and makes a directional prediction.

The null hypothesis (H0) states that there is no effect or no relationship between the variables under test; any observed difference is due to chance. The alternative hypothesis (H1 or Ha) states that a real effect or relationship exists. Statistical tests evaluate whether the data provide sufficient evidence to reject H0 in favour of H1. A research question is broader than a hypothesis: "Does fertiliser concentration affect maize growth?" is a question; the hypothesis converts it into a testable prediction with a stated direction or magnitude of effect.

Fill in the blank: The _____ hypothesis (H0) states that there is no effect or difference between groups; statistical tests determine whether there is sufficient evidence to reject it in favour of the alternative hypothesis.

4.1.3 Variables, controls, and replication

An independent variable (IV) is the variable that the experimenter deliberately changes or manipulates (e.g. fertiliser concentration: 0, 50, 100, 200 mg/L). A dependent variable (DV) is what is measured in response to the IV (e.g. shoot height in cm after four weeks). Controlled variables (CVs) are all other factors that could affect the DV but are held constant to ensure that any change in the DV is caused only by the IV (e.g. light intensity, temperature, watering volume, pot size, soil type).

A control group receives no treatment (e.g. 0 mg/L fertiliser) and provides a baseline against which treatment effects are measured. A positive control (a treatment known to produce the expected effect) confirms the experiment is functioning correctly. Replication means repeating each treatment on multiple independent experimental units (e.g. five pots per fertiliser concentration); it accounts for natural biological variation and allows statistical analysis. Without replication, a single result could be a chance event and no valid statistical conclusion can be drawn.

Fill in the blank: _____ means applying each treatment to multiple independent experimental units; it accounts for natural biological variation and is essential for valid statistical analysis of results.

Variable Type	Definition	Example in Fertiliser–Plant Growth Experiment
Independent Variable	The factor deliberately changed or manipulated by the experimenter	Type or amount of fertiliser applied to plants
Dependent Variable	The factor measured or observed as it responds to changes in the independent variable	Plant growth measured as height, leaf number, or biomass
Controlled Variables	Factors kept constant to ensure a fair test	Same plant species, same soil type, equal water supply, identical light exposure, same pot size

Box 4.1: Summary of experimental variables with examples

Pilot questions 4.1

16. List the seven steps of the scientific method in order.
17. State four characteristics of a good scientific hypothesis.
18. Distinguish between the null hypothesis and the alternative hypothesis.
19. For an experiment testing the effect of light intensity on the rate of photosynthesis in Elodea, identify the independent variable, dependent variable, and three controlled variables.
20. Explain why replication is essential in biological experiments.

4.2 Types of Experimental Design

4.2.1 Completely randomised and randomised block designs

A completely randomised design (CRD) assigns experimental units to treatments entirely at random, with no restrictions. It is the simplest design and is appropriate when all experimental

units are relatively homogeneous (e.g. genetically identical cell cultures or uniform seedlings in a growth chamber). The CRD is easy to set up and analyse but is inefficient when there is considerable variation among experimental units, because this variation inflates the experimental error and reduces the power to detect treatment effects.

A randomised complete block design (RCBD) groups experimental units into blocks of similar units before randomising treatments within each block. Blocks account for a known source of variation (e.g. a gradient in soil fertility, proximity to a window, or batch of reagents). Within each block, every treatment appears once, assigned at random. The RCBD reduces experimental error by removing block-to-block variation from the error term, giving a more sensitive test of treatment effects. It is widely used in field trials and greenhouse experiments.

Fill in the blank: In a randomised complete block design, experimental units are grouped into _____ of similar units before treatments are randomly assigned within each group, removing a known source of variation from the experimental error.

4.2.2 Factorial and Latin square designs

A factorial design tests two or more independent variables (factors) simultaneously in all combinations. A 2 x 3 factorial design, for example, has two levels of factor A and three levels of factor B, giving six treatment combinations. Factorial designs are efficient because they test multiple factors in a single experiment and can detect interaction effects (where the effect of one factor depends on the level of another). Example: testing the combined effect of fertiliser (two levels: none, high) and irrigation (three levels: low, medium, high) on crop yield in a single experiment.

A Latin square design controls for two sources of variation simultaneously (rows and columns) by arranging treatments in a square grid such that each treatment appears exactly once in each row and each column. It is used when two gradients of variation exist simultaneously (e.g. a north-south soil fertility gradient and an east-west drainage gradient across a field plot). The Latin square requires the number of treatments to equal the number of rows and columns, limiting its use to situations where treatment number is manageable (typically three to eight treatments).

Fill in the blank: A factorial design can detect _____ effects, which occur when the effect of one factor on the response variable depends on the level at which a second factor is applied; this information cannot be obtained from one-factor-at-a-time experiments.

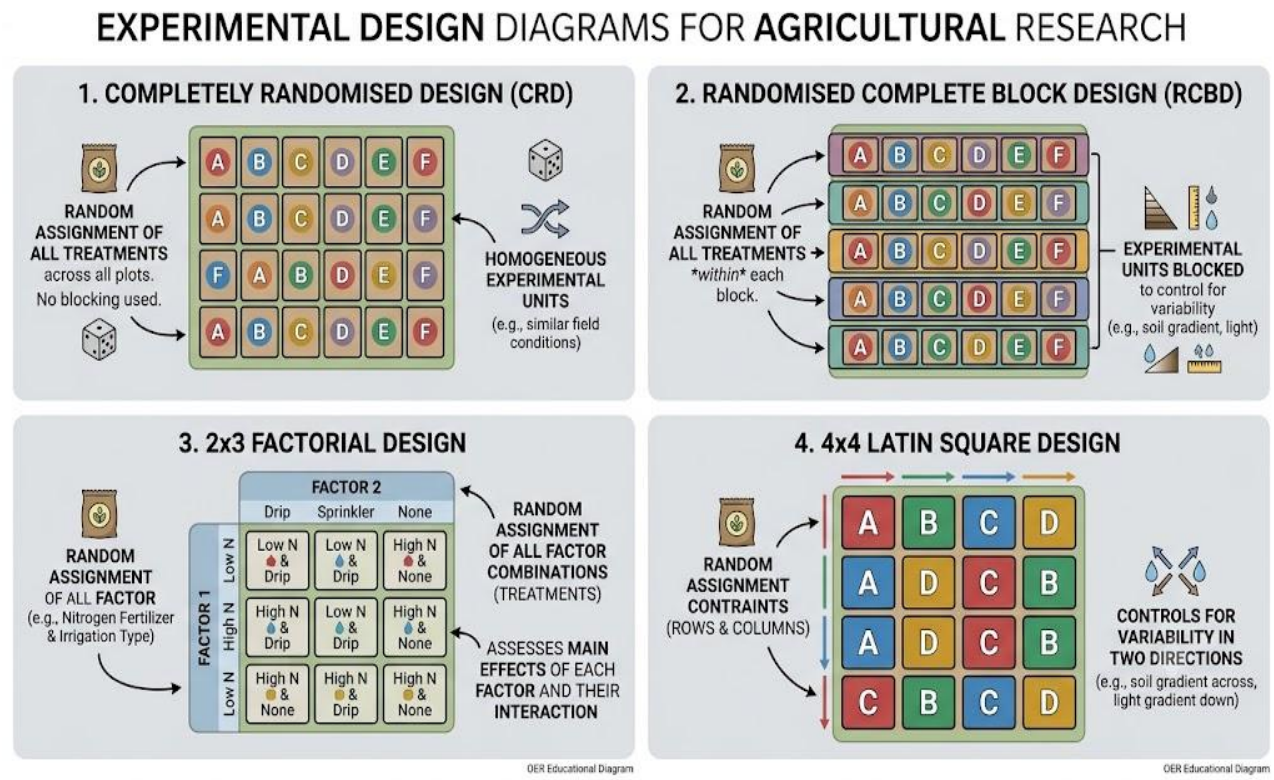

Figure 4.1: Diagrams of four experimental designs

4.2.3 Observational studies and survey design

Not all biological questions can be addressed by manipulative experiments. Observational studies measure variables as they naturally occur without intervention. They are used when manipulation is unethical (e.g. studying disease in humans), impractical (e.g. studying climate effects on ecosystems), or when the aim is to describe natural patterns. Cross-sectional studies measure variables at a single point in time across many individuals (e.g. measuring body mass

index of 500 students). Longitudinal studies follow the same individuals over time (e.g. tracking growth of a cohort of fish over two years).

Survey design involves collecting data from a sample of a population using questionnaires, interviews, or systematic measurements. Key principles: define the target population clearly; use probability-based sampling to avoid bias; ensure the sample size is sufficient for the required statistical power; pre-test the questionnaire or measurement protocol for validity and reliability; and maintain consistency in data collection across all surveyors. Ecological surveys (e.g. quadrat surveys of vegetation cover) follow standardised protocols to ensure comparability across sites and time points.

Fill in the blank: A _____ study measures variables in a sample of subjects at a single point in time without following them over time; a longitudinal study follows the same subjects repeatedly to detect change over a defined period.

Pilot questions 4.2

16. Distinguish between a completely randomised design and a randomised complete block design.
17. Explain the advantage of a factorial design over testing one factor at a time.
18. State the condition required for a Latin square design and give one example of its use.
19. Distinguish between an experimental study and an observational study, and give one example of a situation where only an observational study is ethical.
20. State three key principles of good survey design.

4.3 Data Collection and Sampling Methods

4.3.1 Types of data and measurement scales

Data are classified as qualitative (categorical) or quantitative (numerical). Qualitative data describe categories without numerical value (e.g. flower colour: red, white, yellow; presence or

absence of a disease). Quantitative data are numerical and further divided into: discrete data (whole numbers from counting; e.g. number of eggs in a clutch, number of bacteria colonies on a plate) and continuous data (measurements that can take any value within a range; e.g. body mass in grams, leaf length in mm, absorbance reading).

Measurement scales: nominal scale (categories with no order; e.g. blood groups A, B, AB, O); ordinal scale (categories with a rank order but unequal intervals; e.g. disease severity scored 1 to 5); interval scale (equal intervals but no true zero; e.g. temperature in degrees Celsius); and ratio scale (equal intervals with a true zero; e.g. mass in grams, length in mm, concentration in mol/L). Statistical tests appropriate for data depend on the measurement scale; ratio and interval data allow the widest range of parametric statistical procedures.

Fill in the blank: Data measured on a _____ scale have equal intervals between values and a true zero point, allowing ratios to be calculated; examples include body mass in grams and enzyme activity in units per minute.

4.3.2 Sampling strategies

A sample is a subset of the population measured to make inferences about the whole population. Sampling strategies: random sampling (each member of the population has an equal probability of being selected; minimises bias; requires a complete sampling frame); systematic sampling (units are selected at regular intervals, e.g. every tenth tree along a transect; efficient but may coincide with a periodic pattern in the population); stratified sampling (population is divided into subgroups called strata based on a known characteristic; a random sample is drawn from each stratum proportional to its size; used when subgroups differ in the variable of interest).

Convenience sampling (selecting units that are easily accessible) is not recommended for scientific work as it introduces systematic bias. Cluster sampling (the population is divided into clusters, some clusters are randomly selected, and all units within selected clusters are measured) is used in large-scale ecological or epidemiological surveys when a complete sampling frame is unavailable. Sample size must be large enough to detect the expected effect size at the desired statistical power; too small a sample leads to a high risk of a Type II error (failing to detect a real effect).

Fill in the blank: In _____ sampling, the population is divided into subgroups and a random sample is drawn from each subgroup in proportion to its size; this ensures all subgroups are adequately represented in the final sample.

4.3.3 Sources of error and bias

Random error is unpredictable, affects measurements in both directions, and can be reduced by increasing replication; it is reflected in the variance and standard deviation of the data. Systematic error (bias) consistently pushes measurements in one direction (always too high or always too low); it cannot be reduced by replication and must be eliminated by correcting the source. Sources of systematic error include: miscalibrated instruments (always reading 0.5 mL too high); observer bias (a researcher unconsciously records data consistent with their expectation); and confounding variables (an unmeasured variable that affects the DV and is correlated with the IV).

Minimising error: calibrate all instruments before use; use blind or double-blind protocols where applicable (the observer does not know which treatment group a subject belongs to, preventing observer bias); randomise the order of measurements; standardise procedures with a written protocol; include positive and negative controls; and use adequate replication. Accuracy refers to how close a measurement is to the true value; precision refers to the reproducibility of repeated measurements. High precision with low accuracy indicates systematic bias; high accuracy with low precision indicates large random error.

Fill in the blank: _____ error consistently pushes measurements in one direction and cannot be reduced by replication; it must be eliminated at the source, for example by recalibrating instruments or correcting an observer bias in the measurement protocol.

Pilot questions 4.3

16. Distinguish between discrete and continuous quantitative data with one example each.
17. State the four measurement scales and give one biological example of each.
18. Distinguish between random sampling and stratified sampling.
19. Distinguish between random error and systematic error, and state two methods of reducing each.

20. Explain what a confounding variable is and give one example from a biological experiment.

4.4 Data Analysis and Interpretation

4.4.1 Descriptive statistics

Descriptive statistics summarise and describe the key features of a data set. Measures of central tendency: mean (arithmetic average; sum of all values divided by n; sensitive to outliers); median (middle value when data are ranked; robust to outliers; preferred for skewed distributions); mode (most frequently occurring value; useful for categorical data). Measures of dispersion: range (maximum minus minimum; simple but sensitive to outliers); variance (average squared deviation from the mean; s^2 = sum of (x - x-bar)^2 / (n-1)); standard deviation (SD; the square root of variance; same units as the data); standard error of the mean (SE = SD / square root of n; estimates how precisely the sample mean estimates the population mean).

A 95% confidence interval (CI) for the mean is approximately mean plus or minus 1.96 x SE; it means that if the experiment were repeated many times, 95% of such intervals would contain the true population mean. Descriptive statistics are always reported alongside the raw data in biological reports; the choice between mean plus or minus SD (describing spread within the sample) and mean plus or minus SE (describing precision of the mean estimate) depends on the purpose of the table or figure.

Fill in the blank: The standard error of the mean (SE) is calculated by dividing the standard deviation by the square root of _____; it estimates how precisely the sample mean estimates the true population mean.

4.4.2 Inferential statistics and hypothesis testing

Inferential statistics use sample data to make conclusions about the population and to test hypotheses. The p-value is the probability of obtaining the observed result (or a more extreme one) if the null hypothesis were true. A p-value less than the significance level (usually 0.05) leads to rejection of H0; the result is described as statistically significant. A p-value above 0.05

means insufficient evidence to reject H0 (not proven false); it does not prove H0 is true. A Type I error is rejecting H0 when it is actually true (false positive); a Type II error is failing to reject H0 when it is actually false (false negative).

Common statistical tests in biology: Student's t-test (compares means of two groups; parametric; assumes normality and equal variances); one-way ANOVA (compares means of three or more groups; followed by post-hoc tests such as Tukey's HSD to identify which pairs differ); chi-squared test (tests whether observed frequencies differ from expected frequencies; used for categorical data); Mann-Whitney U test (non-parametric alternative to t-test when data are not normally distributed); Pearson or Spearman correlation (measures the strength of association between two variables). The choice of test depends on the data type, distribution, and the question being asked.

Fill in the blank: A p-value less than _____ (the conventional significance level) leads to rejection of the null hypothesis; the result is described as statistically significant, meaning the observed difference is unlikely to be due to chance alone.

4.4.3 Graphical presentation and interpretation of results

Graphs communicate data patterns more efficiently than tables. Choice of graph: bar chart (comparing means of discrete groups; error bars show SD or SE); line graph (showing change over time or a continuous IV; points connected by lines); scatter plot (showing relationship between two continuous variables; each data point is plotted individually; a line or curve of best fit may be added); histogram (showing the frequency distribution of a continuous variable, divided into class intervals); and pie chart (showing proportions of a whole; limited use in scientific publications).

Graph conventions: x-axis carries the independent variable; y-axis carries the dependent variable; axes must be labelled with the variable name and units; the graph must have a title (or figure caption below); scales must start at zero unless a break is clearly indicated; error bars must be defined in the legend (SD, SE, or 95% CI); data points in a scatter plot should not be connected unless there is a biological justification for assuming a continuous relationship.

Interpreting graphs: describe the overall trend; identify any maximum or minimum; note anomalies; link patterns to the biological explanation.

Fill in the blank: In a correctly drawn graph, the _____ variable is plotted on the x-axis and the dependent variable is plotted on the y-axis; both axes must be labelled with the variable name and its units of measurement.

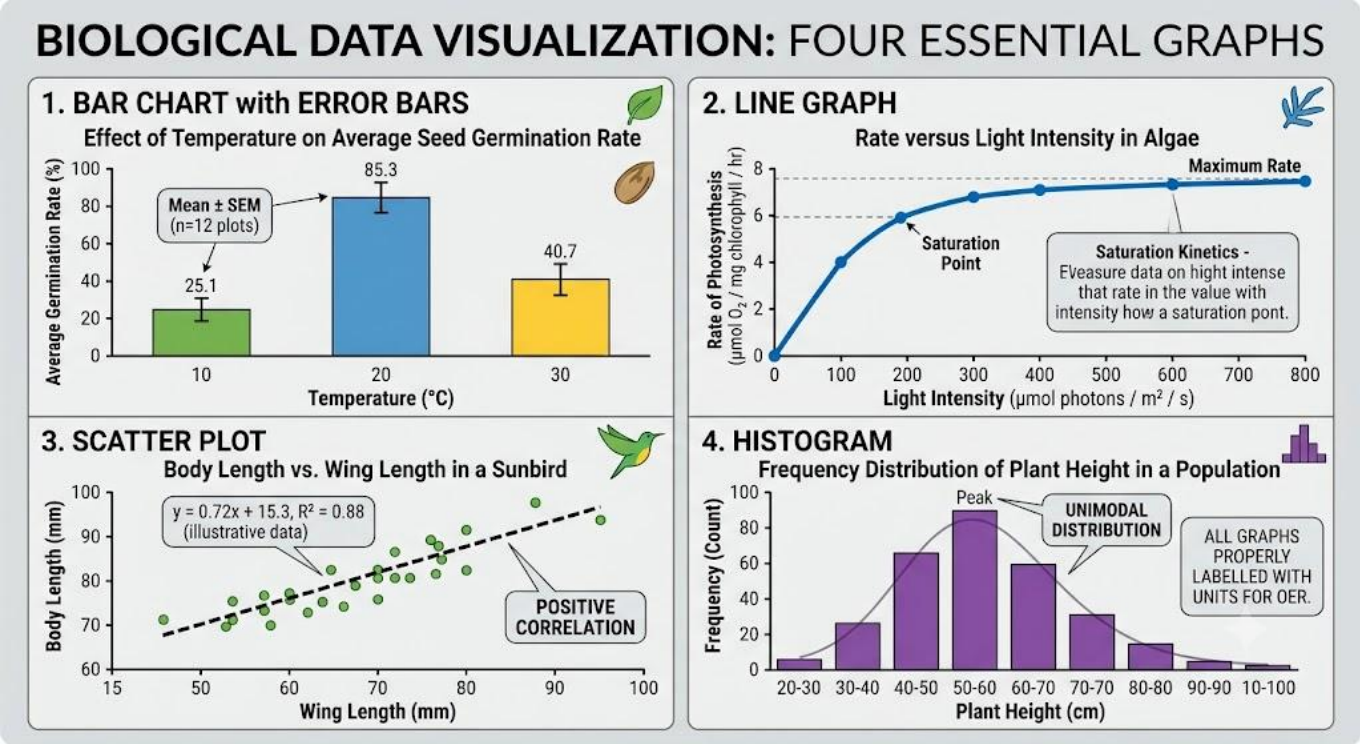

Figure 4.2: Examples of the four main graph types used in biological data presentation

Pilot questions 4.4

16. Distinguish between mean, median, and mode; state when each is the most appropriate measure of central tendency.
17. Explain what a p-value represents and state the conventional significance level used in biology.
18. Distinguish between a Type I error and a Type II error in hypothesis testing.
19. State which statistical test you would use to compare the means of three groups of continuous normally distributed data, and explain why.
20. State five conventions that must be followed when drawing a scientific graph.

Series Summary

This Series covered the principles of experimental design in biology. The scientific method proceeds from observation through hypothesis, experiment, analysis, and conclusion; peer review validates findings. A good hypothesis is testable, falsifiable, and specifies a null (H0) and alternative (H1) form. Independent, dependent, and controlled variables must be identified; control groups and replication are essential. The four main experimental designs are CRD (homogeneous units), RCBD (blocks remove one source of variation), factorial (tests multiple factors and detects interactions), and Latin square (controls two simultaneous gradients).

Observational and survey studies are used when manipulation is impractical or unethical. Data are qualitative or quantitative (discrete or continuous) and measured on nominal, ordinal, interval, or ratio scales. Sampling may be random, systematic, stratified, or cluster. Random error is reduced by replication; systematic error must be eliminated at source. Descriptive statistics (mean, SD, SE, CI) summarise data; inferential tests (t-test, ANOVA, chi-squared) evaluate hypotheses using p-values. Graphs (bar, line, scatter, histogram) present results visually following strict conventions. These skills address SLOs 4.1 to 4.7.

Glossary of Terms

- **Alternative hypothesis (H1):** the hypothesis that a real effect or relationship exists between variables; accepted when the null hypothesis is rejected at the chosen significance level.
- **ANOVA (Analysis of Variance):** a parametric statistical test comparing the means of three or more groups simultaneously; produces an F-statistic and p-value to determine whether group means differ significantly.
- **Bias:** a systematic tendency to over- or under-estimate a true value; caused by faulty measurement, non-random sampling, or observer expectation; cannot be reduced by replication.
- **Confidence interval (CI):** a range of values calculated from sample data within which the true population parameter is expected to fall with a specified probability (typically 95%).
- **Confounding variable:** an unmeasured variable that is correlated with both the independent and dependent variables, potentially producing a spurious association between them.

- **Control group:** a group that receives no treatment (or a standard reference treatment); provides a baseline against which the effect of the experimental treatment is compared.
- **Dependent variable (DV):** the variable measured as the response to the independent variable; plotted on the y-axis of a graph.
- **Factorial design:** an experimental design that tests two or more factors simultaneously in all combinations, enabling detection of interaction effects between factors.
- **Hypothesis:** a specific, testable, and falsifiable statement proposing a relationship between variables; forms the basis of an experiment.
- **Independent variable (IV):** the variable deliberately manipulated by the experimenter; plotted on the x-axis of a graph.
- **Interaction effect:** in a factorial design, the combined effect of two factors that is greater or smaller than the sum of their individual effects; detectable only when both factors are varied simultaneously.
- **Latin square design:** an experimental design arranged as a square grid in which each treatment appears exactly once in each row and each column, controlling for two simultaneous sources of variation.
- **Null hypothesis (H0):** the hypothesis that there is no real effect or difference between groups; any observed difference is due to chance; the hypothesis tested by statistical analysis.
- **p-value:** the probability of obtaining the observed data (or more extreme results) if the null hypothesis were true; a p-value below 0.05 is conventionally taken as evidence to reject H0.
- **Randomised complete block design (RCBD):** an experimental design in which experimental units are grouped into blocks of similar units before treatments are randomly assigned within each block; reduces experimental error by removing block-to-block variation.
- **Replication:** the application of each treatment to multiple independent experimental units; accounts for biological variation and is essential for valid statistical analysis.
- **Standard deviation (SD):** the square root of the variance; measures the spread of individual data values around the mean; expressed in the same units as the data.
- **Standard error (SE):** SD divided by the square root of sample size (n); estimates the precision of the sample mean as an estimate of the population mean; decreases as n increases.
- **Stratified sampling:** a sampling strategy in which the population is divided into subgroups (strata) and a random sample is drawn from each stratum, ensuring all subgroups are represented in proportion to their size.
- **Type I error:** rejecting the null hypothesis when it is actually true (false positive); its probability equals the significance level (alpha, usually 0.05).

- **Type II error:** failing to reject the null hypothesis when it is actually false (false negative); reduced by increasing sample size and statistical power.

Concept Check Questions [Series 4]

Objective Questions

16. The _____ hypothesis (H0) states that there is no effect or difference between groups; it is the hypothesis evaluated by statistical tests. (Linked to SLO 4.2)
17. _____ means applying each treatment to multiple independent experimental units to account for biological variation and enable statistical analysis. (Linked to SLO 4.3)
18. In a randomised complete block design, experimental units are grouped into _____ of similar units before treatments are randomly assigned. (Linked to SLO 4.4)
19. The standard error of the mean (SE) is calculated by dividing the standard deviation by the square root of _____. (Linked to SLO 4.7)
20. A p-value below _____ is the conventional significance threshold at which H0 is rejected and the result is described as statistically significant. (Linked to SLO 4.7)

Short-Answer Questions

21. State four characteristics of a good scientific hypothesis. (Linked to SLO 4.2)
22. Define independent variable, dependent variable, and controlled variable and give one example of each. (Linked to SLO 4.3)
23. Distinguish between the four measurement scales (nominal, ordinal, interval, ratio) with one example each. (Linked to SLO 4.5)
24. Distinguish between random error and systematic error and state one method of reducing each. (Linked to SLO 4.6)
25. State five conventions that must be followed when drawing a scientific graph. (Linked to SLO 4.7)

Long-Answer Questions

20. Describe the scientific method; explain the role of hypothesis, null hypothesis, variables, controls, and replication in a biological experiment. (Linked to SLOs 4.1, 4.2, 4.3)
21. Describe and compare the completely randomised, randomised block, factorial, and Latin square designs; state the conditions under which each is appropriate. (Linked to SLO 4.4)
22. Explain the types of data and sampling strategies used in biology; describe how to analyse data using descriptive and inferential statistics; and state the conventions for graphical presentation of results. (Linked to SLOs 4.5, 4.6, 4.7)

Answers to Concept Check Questions [Series 4]

Objective Questions

16. Null.
17. Replication.
18. Blocks.
19. n (sample size).
20. 0.05.

Short-Answer Questions

21. Testable (can be investigated by experiment or observation); falsifiable (can in principle be shown to be false); based on prior knowledge or observation; makes a specific directional prediction; clearly identifies the variables involved.
22. Independent variable (IV): deliberately manipulated by the experimenter; e.g. fertiliser concentration. Dependent variable (DV): measured as the response to the IV; e.g. shoot height. Controlled variable (CV): held constant to prevent it confounding the results; e.g. temperature.
23. Nominal: categories with no order (e.g. blood groups A, B, AB, O). Ordinal: ranked categories with unequal intervals (e.g. disease severity 1 to 5). Interval: equal intervals, no true zero (e.g. temperature in Celsius). Ratio: equal intervals with a true zero (e.g. body mass in grams).

24. Random error: unpredictable variation in both directions; reduced by increasing replication. Systematic error: consistent bias in one direction; eliminated by recalibrating instruments or correcting the measurement protocol.
25. IV on x-axis; DV on y-axis; both axes labelled with variable name and units; scale starting at zero (or break clearly shown); title or figure caption; error bars defined in the legend.

Long-Answer Questions

20. Scientific method: observation; question; hypothesis; experimental design; data collection; analysis; conclusion; peer review validates findings; conclusions are provisional and reproducible. Hypothesis: specific, testable, falsifiable; predicts a directional outcome. Null hypothesis (H0): no effect; tested statistically; rejected if p is below 0.05. Alternative hypothesis (H1): a real effect exists; accepted when H0 is rejected. Variables: IV (manipulated; e.g. fertiliser level); DV (measured response; e.g. shoot height); CV (held constant; e.g. temperature, light, watering). Control group: receives no treatment; provides a baseline. Replication: multiple units per treatment; accounts for biological variation; without replication, statistical analysis is invalid.
21. CRD: treatments assigned entirely at random; appropriate for homogeneous units (e.g. cell cultures); simple but sensitive to unit variation. RCBD: units grouped into blocks of similar units; treatments randomised within each block; removes block variation from error; used in field trials and greenhouse experiments where a gradient exists. Factorial: tests two or more factors in all combinations simultaneously; efficient; detects interaction effects; e.g. fertiliser x irrigation on crop yield. Latin square: each treatment appears once in every row and column of a square grid; controls two simultaneous gradients (e.g. north-south and east-west soil variation); requires equal number of treatments, rows, and columns.
22. Data types: qualitative (categorical); quantitative (discrete: counting; continuous: measurement). Scales: nominal (no order; blood groups); ordinal (ranked; disease severity); interval (equal intervals, no true zero; Celsius); ratio (equal intervals, true zero; mass in grams). Sampling: random (equal probability; minimises bias); systematic (regular intervals; efficient); stratified (proportional from subgroups); cluster (groups selected, all within measured). Error: random (replication reduces); systematic (recalibration eliminates). Descriptive statistics: mean (sum/n); median (middle ranked value); mode (most frequent); SD (spread of data); SE (precision of mean = SD/sqrt n); 95% CI (mean plus or minus 1.96 x SE). Inferential tests: t-test (two groups; parametric); ANOVA (three or more groups; parametric; followed by post-hoc test); chi-squared (categorical data); Mann-Whitney U (non-parametric). Graph conventions: IV on x-axis; DV on

y-axis; labelled with units; scale from zero; title; error bars defined; data points not connected without biological justification.

Answers to Pilot Questions [Series 4]

Part 4.1

16. (1) Observation; (2) question formulation; (3) hypothesis formulation; (4) experimental design; (5) data collection; (6) data analysis; (7) conclusion and communication.
17. Based on prior knowledge or observation; testable (can be investigated); falsifiable (can in principle be disproved); makes a specific directional prediction about the relationship between variables.
18. Null hypothesis (H0): there is no effect or difference between groups; any observed difference is due to chance. Alternative hypothesis (H1): a real effect or relationship exists between the variables. Statistical tests evaluate whether data provide sufficient evidence to reject H0.
19. Independent variable: light intensity (e.g. 0, 500, 1000, 2000, 4000 lux). Dependent variable: rate of photosynthesis measured as volume of O2 produced per minute. Three controlled variables: temperature of water; CO2 concentration (sodium bicarbonate concentration); length of Elodea strand used.
20. Biological organisms show natural variation; a single measurement per treatment could be due to chance rather than a true treatment effect. Replication produces multiple data points per treatment, allowing calculation of means, variance, and standard error, and enabling statistical tests to determine whether differences between treatments exceed what would be expected from chance variation alone.

Part 4.2

16. CRD: treatments assigned entirely at random to homogeneous units; simple but inefficient if units vary. RCBD: units are first grouped into blocks of similar units; treatments are then randomised within each block; block variation is removed from the error term, giving a more sensitive test; appropriate when a known source of variation exists (e.g. a soil fertility gradient, shelf position in a growth chamber).

17. A factorial design tests multiple factors simultaneously in all combinations, which is more efficient than separate experiments. It also detects interaction effects (where the effect of one factor depends on the level of another); these interactions are completely missed when only one factor is varied at a time. For example, the combined effect of fertiliser and irrigation on crop yield may be greater than the sum of their individual effects.
18. Condition: the number of treatments must equal the number of rows and the number of columns (i.e. a square arrangement). Example: testing four crop varieties (A, B, C, D) across a field with a north-south soil moisture gradient and an east-west slope gradient; each variety appears once in each row and each column, controlling for both gradients simultaneously.
19. Experimental study: the researcher deliberately manipulates an IV and measures the effect on a DV; can establish cause and effect. Observational study: the researcher measures variables as they naturally occur without manipulation; can identify associations but not prove causation. Example where only observational is ethical: studying the health effects of cigarette smoking in humans; randomly assigning people to smoke would be unethical.
20. Define the target population clearly; use probability-based (random or stratified) sampling to avoid bias; ensure the sample size is sufficient for the required statistical power; pre-test the questionnaire or measurement protocol for validity and reliability; maintain consistency in data collection across all surveyors and sites.

Part 4.3

16. Discrete data: whole number values obtained by counting; e.g. number of eggs in a clutch (3, 4, 5; cannot be 3.7). Continuous data: can take any value within a range, obtained by measurement; e.g. body mass of a fish in grams (23.7 g, 24.1 g).
17. Nominal: categories with no order; e.g. blood groups (A, B, AB, O). Ordinal: ranked categories with unequal intervals; e.g. disease severity scored 1 to 5. Interval: equal intervals, no true zero; e.g. temperature in degrees Celsius. Ratio: equal intervals with a true zero; e.g. body mass in grams or enzyme activity in units per minute.
18. Random sampling: every member of the population has an equal probability of selection; minimises bias but requires a complete sampling frame. Stratified sampling: population is divided into subgroups (strata) based on a known characteristic; a random sample is drawn from each stratum proportional to its size; ensures all subgroups are represented and is used when subgroups differ in the variable of interest.

19. Random error: unpredictable variation in both directions; reduced by (1) increasing the number of replicates and (2) using more precise instruments. Systematic error: consistent bias in one direction; eliminated by (1) recalibrating instruments before use and (2) using blind protocols to prevent observer bias.
20. A confounding variable is an unmeasured factor that is correlated with both the IV and the DV, creating a spurious association. Example: in a study of the effect of shade on plant growth, if shaded plots also happen to receive more water (due to reduced evaporation), then water availability is a confounding variable; the observed growth difference could be due to water, not shade.

Part 4.4

16. Mean: sum of values divided by n; best for symmetrical distributions without outliers. Median: middle value when ranked; best for skewed distributions or data with outliers (e.g. income, survival times). Mode: most frequent value; best for categorical data or when the most common response is required (e.g. modal disease score in a clinical survey).
17. The p-value is the probability of obtaining the observed result (or a more extreme one) if the null hypothesis were true. In biology, the conventional significance level is 0.05; if p is below 0.05, H0 is rejected and the result is considered statistically significant, meaning the probability of the result occurring by chance alone is less than 5%.
18. Type I error (false positive): H0 is rejected when it is actually true; occurs with probability equal to the significance level (0.05); means concluding there is an effect when there is none. Type II error (false negative): H0 is not rejected when it is actually false; means missing a real effect; reduced by increasing sample size.
19. One-way ANOVA (Analysis of Variance); used because it compares the means of three or more groups simultaneously in a single test; applying multiple t-tests inflates the Type I error rate. If ANOVA gives a significant F-statistic (p below 0.05), a post-hoc test (e.g. Tukey's HSD) identifies which specific pairs of group means differ significantly.
20. IV on x-axis; DV on y-axis; both axes labelled with variable name and units; scale starting at zero (or break clearly indicated); figure caption below the graph; error bars present and defined in the legend (SD, SE, or 95% CI); data points not connected unless a continuous relationship is biologically justified.

References and Attributions [Series 4]

Textual References (Books and External Sources)

- Campbell, N. A., & Reece, J. B. (2008). Biology (8th ed.). Pearson Benjamin Cummings.
- Fowler, J., Cohen, L., & Jarvis, P. (1998). Practical statistics for field biology (2nd ed.). Wiley.
- Montgomery, D. C. (2017). Design and analysis of experiments (9th ed.). Wiley.
- Sokal, R. R., & Rohlf, F. J. (2012). Biometry: The principles and practice of statistics in biological research (4th ed.). W.H. Freeman.

Figures, Plates, Tables, and Boxes

- Box 4.1: Summary of experimental variable types with examples Attribution: AI generated. Suggested OER search string: "experimental design variables independent dependent controlled control replication table OER".
- Figure 4.1: Diagrams of four experimental designs Attribution: AI generated. Suggested OER search string: "completely randomised design randomised block factorial Latin square experimental design diagram OER".
- Figure 4.2: Examples of the four main graph types used in biological data presentation Attribution: AI generated. Suggested OER search string: "biological data graphs bar chart line graph scatter plot histogram examples labelled OER".

Course code: BIO 204

Course title: Biological Techniques

Course credit load: 2 Units

Series 5: Report Writing and Presentation Skills

- **Part 5.1: Structure and Components of a Scientific Report**
    - Sub Part 5.1.1: Title, abstract, and introduction
    - Sub Part 5.1.2: Materials and methods, and results
    - Sub Part 5.1.3: Discussion, conclusion, and references
- **Part 5.2: Scientific Writing Style and Referencing**
    - Sub Part 5.2.1: Language and style in scientific writing
    - Sub Part 5.2.2: In-text citation and reference lists
    - Sub Part 5.2.3: Academic integrity and avoiding plagiarism
- **Part 5.3: Oral Presentation Skills**
    - Sub Part 5.3.1: Structuring an oral presentation
    - Sub Part 5.3.2: Designing and using visual aids
    - Sub Part 5.3.3: Delivery, body language, and handling questions
- **Part 5.4: Poster Presentations and Science Communication**
    - Sub Part 5.4.1: Designing a scientific poster
    - Sub Part 5.4.2: Communicating science to non-specialist audiences
    - Sub Part 5.4.3: Ethics and responsibility in science communication

Introduction

The ability to communicate scientific findings clearly and accurately is as important as the ability to generate them. A discovery that is poorly written, misrepresented, or never presented has no scientific value. Scientists communicate their work through written reports and journal articles, oral presentations at seminars and conferences, poster presentations, and increasingly through digital and public media. Each format demands specific skills that must be learnt and practised.

This Series covers the structure and content of a formal scientific report, the conventions of scientific writing and referencing, the skills required for effective oral and poster presentations, and the principles of responsible science communication to specialist and non-specialist

audiences. These skills integrate all the practical and analytical techniques from Series 1 to 4 into a complete workflow from experiment to dissemination.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- SLO 5.1 describe the standard sections of a scientific report and state the purpose and content of each (Linked to Part 5.1),
- SLO 5.2 write a materials and methods section and a results section following scientific conventions (Linked to Part 5.1),
- SLO 5.3 apply the conventions of scientific writing style and correctly cite sources using APA or Vancouver format (Linked to Part 5.2),
- SLO 5.4 define plagiarism and describe strategies for maintaining academic integrity in scientific writing (Linked to Part 5.2),
- SLO 5.5 structure and deliver an effective oral presentation of scientific findings (Linked to Part 5.3),
- SLO 5.6 design a scientific poster that communicates key findings clearly and effectively (Linked to Part 5.4), and
- SLO 5.7 explain the principles of responsible science communication to non-specialist audiences and describe the ethical obligations of scientists (Linked to Part 5.4).

5.1 Structure and Components of a Scientific Report

5.1.1 Title, abstract, and introduction

The title must be concise, informative, and specific; it should identify the organism, the variable studied, and the response measured (e.g. "Effect of nitrogen fertiliser concentration on shoot growth of Zea mays seedlings"). Avoid vague titles such as "An experiment on plants." The abstract is a self-contained summary of the entire report, typically 150 to 250 words, written last

but placed first. It must state: the aim; the methods used; the key results (with numerical values); and the main conclusion. The abstract must not contain information absent from the main report.

The introduction provides the scientific background needed to understand the study. It should: review relevant published literature (with citations); identify the gap in knowledge that the study addresses; state the aim of the study; and state the hypothesis being tested. The introduction moves from broad context to specific focus (the "funnel" structure): start with the general topic, narrow to the specific problem, and end with the aim and hypothesis. It is written in the present tense for established facts and the past tense for the specific study.

Fill in the blank: The abstract is typically 150 to 250 words and must state the aim, the methods, the key _____ (with numerical values), and the main conclusion; it is written last but placed at the beginning of the report.

5.1.2 Materials and methods, and results

The materials and methods section describes exactly what was done and what was used, in sufficient detail for another competent scientist to replicate the experiment. It must include: the organisms or materials used (species, source, age, condition); equipment and reagents (manufacturer, grade, concentration); the experimental design (treatments, controls, replication); the measurement procedure (how the DV was measured, the instrument used, the units); and the statistical analysis applied. It is written in the past tense, passive voice (e.g. "Seedlings were watered daily with 50 mL of distilled water"), and third person.

The results section presents the data objectively without interpretation. It must include: a concise text description of the main findings; tables and figures (graphs) with self-explanatory titles and captions; and reference to statistical outcomes (test used, test statistic, degrees of freedom, p-value). The text must direct the reader to each table or figure (e.g. "Mean shoot height increased with fertiliser concentration (Figure 1); differences between treatments were statistically significant (one-way ANOVA, F3,16 = 18.4, p less than 0.001)"). Raw data are placed in an appendix; means and statistics are in the main text.

Fill in the blank: The materials and methods section is written in the past tense, _____ voice, and third person so that another scientist could replicate the experiment exactly from the description given.

5.1.3 Discussion, conclusion, and references

The discussion interprets the results in the context of the hypothesis and existing literature. It should: state whether the hypothesis was supported or rejected and why; explain the biological mechanisms behind the results; compare the findings with those of previously published studies (with citations); discuss sources of error or limitations of the experimental design; and suggest directions for future research. The discussion moves from specific findings back to the broad context (the inverse funnel). It is the most intellectually demanding section and must not simply repeat the results.

The conclusion is a brief paragraph (three to five sentences) summarising the main finding, its significance, and any key limitation. It answers the original research question directly. The reference list contains the full bibliographic details of every source cited in the report, formatted in the required style (APA, Vancouver, or the journal's house style). No source should appear in the reference list if it is not cited in the text, and no in-text citation should lack a corresponding entry in the reference list.

Fill in the blank: The discussion section moves from the specific findings back to the broad context; it must state whether the _____ was supported or rejected, explain the biological mechanisms, compare with published literature, and discuss limitations and future directions.

Section	Purpose	Tense	Key Content
Title	Clearly describes the study focus	Present	Concise, specific, includes keywords
Abstract	Summarizes the entire report	Past (methods/results), Present (conclusion)	Objectives, methods, main findings, significance
Introduction	Provides background and rationale	Present	Problem statement, literature context, research question/hypothesis

Section	Purpose	Tense	Key Content
Methods	Explains how the study was conducted	Past	Study design, materials, procedures, data collection
Results	Presents findings without interpretation	Past	Data, figures, tables, statistical outcomes
Discussion	Interprets results and connects to literature	Present	Analysis, implications, limitations, comparison with prior studies
Conclusion	Summarizes contributions and significance	Present	Key findings, broader impact, recommendations, future research
References	Credits sources used	Present	Full citations in required format (APA, MLA, etc.)

Box 5.1: Summary of the sections of a scientific report, their purpose, tense, and key content

Pilot questions 5.1

21. State the four items of information that must be included in an abstract.
22. Describe the "funnel" structure of an introduction and state what its final sentence should contain.
23. State three items that must be included in the materials and methods section.
24. Distinguish between the results section and the discussion section of a scientific report.
25. Explain the relationship between in-text citations and the reference list.

5.2 Scientific Writing Style and Referencing

5.2.1 Language and style in scientific writing

Scientific writing prioritises clarity, precision, and objectivity. Key principles: use simple, direct language; avoid jargon unless it is defined; prefer the passive voice for methods (e.g. "Samples were centrifuged at 3,000 g") but active voice for emphasis in the discussion (e.g. "These results suggest that..."); use the past tense for specific experimental actions; use the present tense for

established facts and for referring to figures and tables (e.g. "Figure 1 shows..."); avoid colloquial expressions, emotional language, or unsubstantiated claims.

Additional conventions: use SI units throughout; write numbers as numerals when accompanied by a unit (e.g. 5 mL, 37 degrees Celsius) but spell out numbers below ten when used without a unit (e.g. "five replicates"); never start a sentence with a numeral; use italic for species names (e.g. Escherichia coli); abbreviate terms after their first full use (e.g. "polymerase chain reaction (PCR)"); and define all symbols, abbreviations, and non-standard terms at first use. Paragraphs should be focused; each paragraph makes one main point, introduced in the first sentence.

Fill in the blank: In scientific writing, numbers accompanied by a unit are written as _____ (e.g. 5 mL, 37 degrees Celsius), but numbers below ten that are used without a unit are spelled out in full (e.g. "five replicates").

5.2.2 In-text citation and reference lists

In-text citations acknowledge the source of information or ideas. APA (author-date) format: cite as (Author, Year) or Author (Year) when the author's name is part of the sentence. For two authors: (Smith & Jones, 2020). For three or more authors: (Smith et al., 2019). For a direct quotation, include the page number: (Smith, 2018, p. 45). Vancouver (numbered) format: sources are cited as superscript numbers or numbers in brackets in the order they first appear in the text; the reference list is numbered in citation order rather than alphabetically.

Reference list formats. APA journal article: Author, A. A., & Author, B. B. (Year). Title of article. Journal Name, Volume(Issue), page range. https://doi.org/... APA book: Author, A. A. (Year). Title of work: Capital letter also for subtitle (Edition). Publisher. Vancouver journal article: Author AA, Author BB. Title of article. Abbreviated Journal Name. Year;Volume(Issue):page range. Every entry must be complete and consistent; the reference list is the reader's route to verifying every claim in the report.

Fill in the blank: In APA format, a work with three or more authors is cited in the text as the first author's surname followed by "_____ al." and the year (e.g. Smith et al., 2021); the full author list appears only in the reference list entry.

5.2.3 Academic integrity and avoiding plagiarism

Plagiarism is presenting another person's words, ideas, data, or images as your own without acknowledgement. It is a serious breach of academic integrity and may result in expulsion, retraction of publications, and damage to a scientist's career. Forms of plagiarism: verbatim copying without quotation marks and citation; paraphrasing another's ideas without citation; self-plagiarism (reusing substantial portions of one's own previously submitted work without disclosure); data fabrication (inventing data); and data falsification (selectively altering data to support a desired conclusion).

Avoiding plagiarism: always cite the source when using another's ideas or data; paraphrase in your own words and still cite; use quotation marks for direct quotations (rare in science); keep clear notes distinguishing your own ideas from those read in sources; use plagiarism detection software (e.g. Turnitin) as a checking tool before submission; and never share your assignment files with other students. Open science practices (pre-registration of study design, sharing of raw data, open-access publication) promote transparency and reduce the opportunity for misconduct.

Fill in the blank: _____ is the invention of data that were never collected; data falsification is the selective alteration of real data to support a desired conclusion; both are serious forms of research misconduct with severe professional and legal consequences.

Pilot questions 5.2

21. State five principles of good scientific writing style.
22. Write an APA-format in-text citation for a journal article with four authors (Smith, Jones, Obi, and Adeyemi) published in 2022.
23. Write a correctly formatted APA reference list entry for a journal article.
24. Define plagiarism and state three forms it can take in a scientific report.
25. Distinguish between data fabrication and data falsification and state the consequences of each.

5.3 Oral Presentation Skills

5.3.1 Structuring an oral presentation

A scientific oral presentation follows the same logical sequence as a written report but compressed into the available time. Standard structure for a 10 to 15 minute research presentation: title slide (title, author name, affiliation, date); introduction (1 to 2 slides: background, gap, aim, hypothesis); methods (1 to 2 slides: design, key procedures; enough for the audience to evaluate the results, not a step-by-step protocol); results (3 to 5 slides: one key finding per slide; use figures rather than tables where possible); discussion (1 to 2 slides: interpretation, comparison with literature, limitations); and conclusion (1 slide: take-home message, future directions). End with an acknowledgements slide and a references slide.

The talk must have a clear narrative arc: "Tell them what you are going to tell them (introduction); tell them (methods and results); tell them what you told them (discussion and conclusion)." Practise to fit within the time limit; overrunning is disrespectful to the audience and the chair. Prepare a one-sentence answer to the most likely questions. Anticipate at least three questions the audience might ask and prepare answers. Never put more information on a slide than you will explain; unread text on slides distracts the audience.

Fill in the blank: The conclusion slide of an oral presentation should state one clear _____ message: what the audience should remember about the study; it should be expressed in plain language in a single sentence or two short bullet points.

5.3.2 Designing and using visual aids

Slides (PowerPoint, Google Slides, or equivalent) are the standard visual aid for scientific presentations. Design principles: use a plain, consistent background (light background with dark text is most legible); limit text to six lines per slide and six words per line (the 6x6 rule); use a minimum font size of 24 pt for body text and 32 pt or more for headings; use high-contrast colour combinations; avoid using decorative clip art or animations that distract from the content; and ensure all figures and tables are large enough to be read from the back of the room.

Effective use of figures: each data figure should appear on its own slide with a brief title at the top and axis labels and units visible; build complex figures incrementally using animations if needed; always describe what the figure shows before asking the audience to interpret it; and state the take-home message of each figure explicitly ("This graph shows that shoot height increased linearly with fertiliser concentration up to 100 mg/L, after which no further increase was seen"). Photographs, diagrams, and videos can replace text when showing a technique, organism, or piece of equipment.

Fill in the blank: The 6x6 rule for slide design recommends a maximum of six lines of text per slide and six _____ per line, keeping slides uncluttered and ensuring the audience focuses on the speaker rather than reading the screen.

5.3.3 Delivery, body language, and handling questions

Effective delivery: speak clearly and at a moderate pace (not too fast, not too slow); vary pitch and emphasis to maintain interest; make eye contact with different parts of the audience rather than reading from notes or facing the screen; use a laser pointer or cursor to direct attention to specific parts of a figure; pause briefly after making an important point to allow the audience to absorb it. Nervousness is normal; preparation and rehearsal are the most effective remedies. A well-rehearsed speaker who knows their material can redirect attention away from any technical difficulties.

Body language: stand upright and face the audience; avoid turning your back to read from the screen; do not block the projected image; use natural hand gestures to emphasise points; avoid distracting habits (jingling keys, clicking a pen, swaying). Handling questions: listen to the full question before answering; rephrase or repeat the question for the whole audience; if you do not know the answer, say so honestly ("That is a very good question; I do not know the answer, but I will find out and follow up"); never argue with the questioner; and thank the audience at the end.

Fill in the blank: When asked a question you cannot answer during the Q&A, the appropriate response is to acknowledge the question honestly and say you will _____ up with the answer rather than attempting to invent an answer or dismissing the question.

21. State the standard sections of a 10 to 15 minute scientific oral presentation in order.
22. State five principles of good slide design.
23. Explain the 6x6 rule for slide design.
24. State three principles of effective oral delivery in a scientific presentation.
25. Describe the correct way to handle a question you cannot answer during the Q&A session.

5.4 Poster Presentations and Science Communication

5.4.1 Designing a scientific poster

A scientific poster presents key findings from a study on a single large sheet (typically A0, 841 x 1189 mm, portrait or landscape orientation). It is designed to be read independently in 3 to 5 minutes and to prompt conversation between the presenter and passing scientists at a conference poster session. A poster must be visually attractive, clearly organised, and readable from one metre away. Standard layout: title and authors at the top (largest text, typically 72 to 90 pt); then columns of content below arranged as Introduction, Methods, Results, Conclusions; with contact details and acknowledgements at the bottom.

Design principles: text should be concise; use bullet points rather than continuous prose; minimum body text size 24 pt; limit to one or two key figures; use high-resolution images only; leave white space between sections to aid readability; use a consistent colour scheme (two to three colours maximum); and ensure the visual flow guides the reader's eye logically from title to conclusions. A QR code linking to the full paper or a supplementary data set is increasingly used to provide additional detail without cluttering the poster surface.

Fill in the blank: A scientific poster should be readable from _____ metre away; body text should be a minimum of 24 pt and the title at least 72 pt to ensure legibility across the width of a conference poster session room.

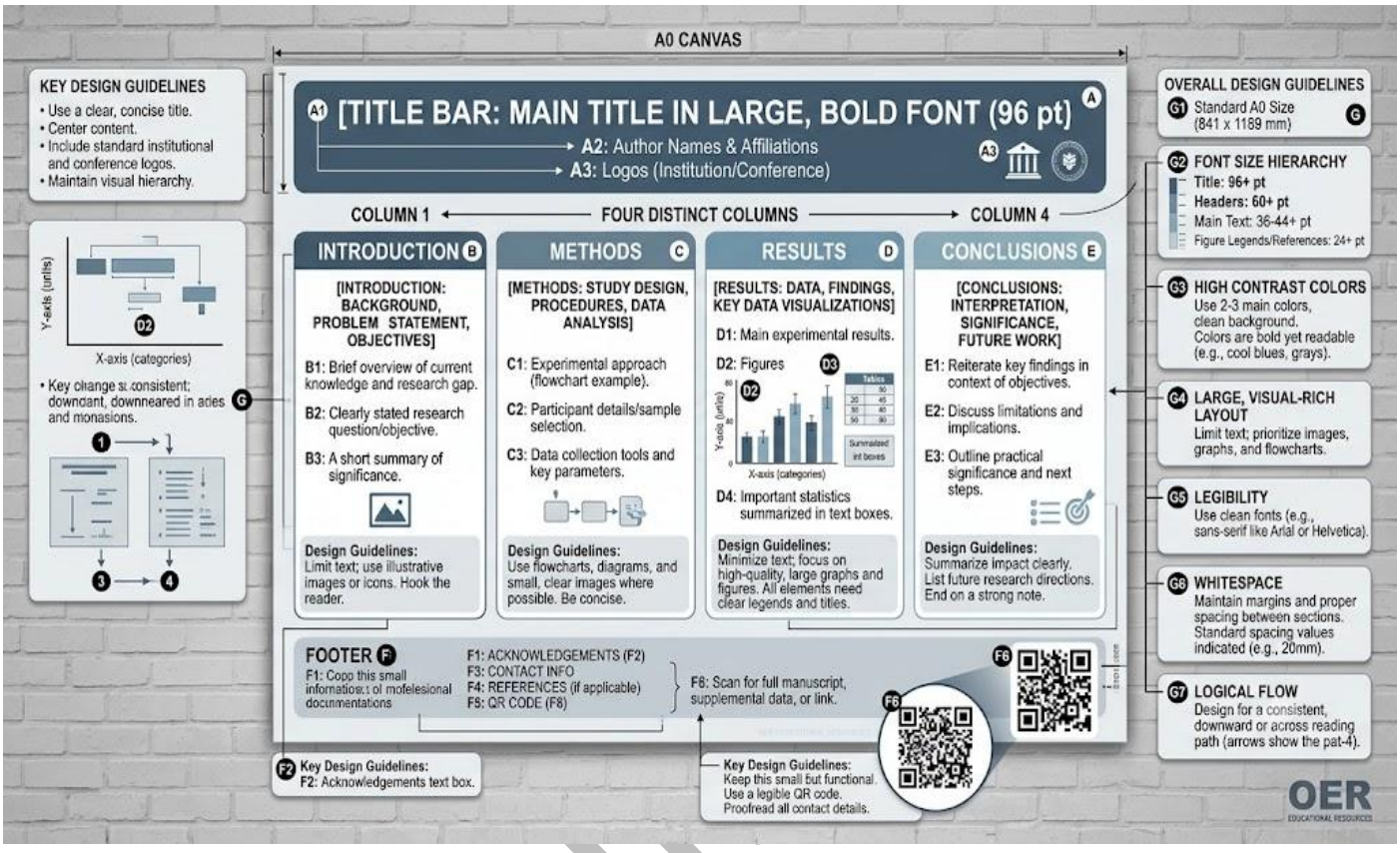

Figure 5.1: Annotated layout of a correctly designed scientific conference poster

5.4.2 Communicating science to non-specialist audiences

Science communication to the public, policy makers, and journalists requires a different approach from specialist scientific writing. Key principles: replace technical jargon with plain language equivalents; use analogies and concrete examples to explain abstract concepts (e.g. "The DNA in a single human cell, if stretched out, would be about two metres long, yet it is coiled to fit into a nucleus visible only under a microscope"); lead with the relevance or significance to everyday life before explaining the mechanism; avoid overstating certainty or hedging so much that findings seem useless.

In Nigeria, effective science communication has practical importance: communicating results of agricultural research to smallholder farmers; explaining vaccine safety to communities with vaccine hesitancy; translating climate research findings into policy recommendations; and informing the public about disease outbreaks. Scientists must tailor their message to the specific

audience, check that the audience understood the key point, and be prepared to answer questions in accessible language. Visuals (photographs, infographics, simple charts) are more effective than text for most non-specialist audiences.

Fill in the blank: When communicating science to non-specialist audiences, technical jargon should be replaced with _____ language equivalents, and abstract concepts should be explained using concrete analogies and examples relevant to the audience's everyday experience.

5.4.3 Ethics and responsibility in science communication

Scientists have an ethical obligation to communicate their findings accurately, honestly, and without distortion. Selective reporting (reporting only results that support the hypothesis, known as publication bias or p-hacking) distorts the scientific record and can cause harm (e.g. reporting only positive drug trial results while suppressing negative ones). Scientists must report null results as well as positive ones. Conflicts of interest (financial, personal, or institutional relationships that could bias interpretation) must be declared in publications and presentations.

Media reporting of science often strips context, overstates certainty, or presents preliminary findings as established fact. Scientists should: issue press releases only for peer-reviewed, published findings; correct media misrepresentations promptly and publicly; engage with science journalists to ensure accurate translation; and avoid using social media to announce unpublished findings as established fact. The principle of informed consent requires that research involving human subjects is only undertaken with participants' full, voluntary, and informed agreement; data must be anonymised where appropriate. All biological research in Nigeria must comply with relevant national and institutional ethics guidelines.

Fill in the blank: _____ bias (also called publication bias) occurs when only studies with positive or significant results are published while negative or null results are suppressed, distorting the overall scientific record and potentially misleading subsequent research and policy.

Pilot questions 5.4

21. State five design principles for a scientific conference poster.

22. State three differences between communicating science to specialists and to a non-specialist audience.
23. Give two examples of why effective science communication is practically important in Nigeria.
24. Explain what publication bias is and state its consequences for science and public policy.
25. State three ethical obligations of a scientist when communicating research findings to the public or through the media.

Series Summary

This Series introduced the essential skills of scientific communication, covering how to write a formal scientific report, deliver an oral presentation, design a research poster, and communicate science to non-specialist audiences. A scientific report is organised into eight sections, each with a specific purpose: the title identifies the study; the abstract (150 to 250 words) provides a concise summary of the entire work; the introduction builds context using a funnel structure that moves from the broad topic to the specific research question; the materials and methods section describes exactly how the study was conducted, written in the past passive voice so that another scientist could repeat the work; the results section presents data objectively using figures, tables, and appropriate statistics without interpretation; the discussion interprets the findings, compares them with the existing literature, and explains whether the hypothesis was supported; the conclusion briefly states what the study found and why it matters; and the references list all sources cited. Throughout, scientific writing must be clear, precise, and objective, using correct SI units, consistent abbreviations, and appropriate tense in each section.

Ethical conduct in scientific communication is as important as technical skill. In-text citations and reference lists must follow a recognised format such as APA or Vancouver; plagiarism and data fabrication are serious violations that can end a scientific career. Oral presentations follow the same logical structure as a written report and require well-designed slides (obeying the 6×6 rule with text no smaller than 24 point), confident delivery with eye contact and clear speech, thorough rehearsal, and honest handling of audience questions. Scientific posters (A0 size) present key findings visually in a format a visitor can read independently in 3 to 5 minutes, using bullet points, large readable text, and one or two carefully chosen figures. When communicating

with non-specialist audiences, scientists must use plain language, helpful analogies, and relevant everyday examples to make their findings accessible without sacrificing accuracy. Selective reporting, publication bias, and undeclared conflicts of interest are all recognised as ethical violations that undermine public trust in science.

Glossary of Terms

- **Abstract:** a self-contained summary (150 to 250 words) of an entire scientific report, stating the aim, methods, key results, and conclusion; written last, placed first.
- **Academic integrity:** the commitment to honesty, trust, fairness, and responsibility in all academic and research activities; violated by plagiarism, data fabrication, and falsification.
- **APA format:** American Psychological Association referencing style using author-date in-text citations (Author, Year) and an alphabetical reference list; widely used in biology and social sciences.
- **Conflict of interest:** a financial, personal, or institutional relationship that could bias a scientist's interpretation or reporting of research findings; must be declared in publications and presentations.
- **Data fabrication:** inventing data that were never collected; a form of research misconduct with severe professional and legal consequences.
- **Data falsification:** the selective alteration of real data to produce a desired result; a form of research misconduct distinct from fabrication.
- **Discussion:** the section of a scientific report that interprets the results in the context of the hypothesis and published literature; explains mechanisms, discusses limitations, and suggests future directions.
- **Funnel structure:** the organisational pattern of a scientific introduction, moving from broad background to the specific research question, aim, and hypothesis.
- **In-text citation:** a brief reference in the body of a report acknowledging the source of information or ideas; in APA format: (Author, Year); links to the full entry in the reference list.
- **Informed consent:** voluntary agreement by a research participant, given after receiving full information about the nature, risks, and benefits of the study; required for all human subjects research.

- **Materials and methods:** the section of a scientific report describing in detail what was used and what was done, enabling replication; written in past tense, passive voice, third person.
- **Passive voice:** a grammatical construction in which the subject of the sentence receives the action (e.g. "Samples were centrifuged"); standard in the materials and methods section of a scientific report.
- **Peer review:** evaluation of a scientific manuscript by independent experts in the field before publication; the primary mechanism for quality control in the scientific literature.
- **Plagiarism:** presenting another person's words, ideas, data, or images as one's own without proper acknowledgement; a serious breach of academic integrity.
- **Publication bias:** the tendency for journals and researchers to publish positive or significant results while suppressing null or negative results, distorting the scientific record.
- **Reference list:** a complete, formatted list of all sources cited in a report; every in-text citation must have a corresponding entry, and vice versa.
- **Results section:** the section of a scientific report presenting the data objectively, including text descriptions, figures, tables, and statistical outcomes, without interpretation.
- **Science communication:** the practice of conveying scientific knowledge and findings to specialist and non-specialist audiences through written, oral, visual, and digital media.
- **Self-plagiarism:** reusing substantial portions of one's own previously submitted or published work in a new submission without disclosure; considered a form of academic misconduct.
- **Vancouver format:** a numbered referencing style in which sources are cited as sequential numbers in the text and listed in citation order in the reference list; standard in biomedical and clinical sciences.

Concept Check Questions [Series 5]

Objective Questions

21. The abstract is typically 150 to 250 words and must state the aim, methods, key _____, and conclusion. (Linked to SLO 5.1)
22. The materials and methods section is written in the past tense, _____ voice, and third person. (Linked to SLO 5.2)

23. In APA format, a work with three or more authors is cited in the text as the first author followed by "_____ al." and the year. (Linked to SLO 5.3)
24. The 6x6 rule for slide design recommends a maximum of six lines of text per slide and six _____ per line. (Linked to SLO 5.5)
25. _____ bias occurs when only significant results are published while null results are suppressed, distorting the scientific record. (Linked to SLO 5.7)

Short-Answer Questions

26. State the eight sections of a scientific report in order and give the purpose of each. (Linked to SLO 5.1)
27. State five principles of scientific writing style. (Linked to SLO 5.3)
28. Define plagiarism and state three forms it can take. (Linked to SLO 5.4)
29. State five design principles for a scientific conference poster. (Linked to SLO 5.6)
30. State three ethical obligations of scientists when communicating research findings. (Linked to SLO 5.7)

Long-Answer Questions

23. Describe the structure and content of a scientific report; explain the purpose and required content of each section and state the appropriate tense and voice for the materials and methods and results sections. (Linked to SLOs 5.1, 5.2)
24. Describe the conventions of scientific writing style, explain APA and Vancouver referencing formats, define plagiarism, and describe strategies for maintaining academic integrity. (Linked to SLOs 5.3, 5.4)
25. Describe the design of an effective oral presentation and a scientific poster; explain the principles of responsible science communication to non-specialist audiences and the ethical obligations of scientists. (Linked to SLOs 5.5, 5.6, 5.7)

Answers to Concept Check Questions [Series 5]

Objective Questions

21. Results (with numerical values).
22. Passive.
23. et.
24. Words.
25. Publication.

Short-Answer Questions

26. Title (identifies organism, variable, response); Abstract (self-contained summary; aim, methods, results, conclusion); Introduction (background, gap, aim, hypothesis; funnel structure); Materials and methods (enables replication); Results (presents data objectively; figures, tables, statistics); Discussion (interprets findings, compares with literature, discusses limitations); Conclusion (brief; answers research question); References (full citations of all sources cited).
27. Use clear, direct language; avoid unnecessary jargon; use passive voice in methods; use past tense for experimental actions; present tense for established facts and figure references; SI units throughout; numbers with units as numerals; species names in italics; define all abbreviations at first use.
28. Plagiarism: presenting another's words, ideas, data, or images as your own without acknowledgement. Three forms: verbatim copying without quotation marks and citation; paraphrasing without citation; data fabrication (inventing data that were never collected).
29. Readable from one metre; minimum 24 pt body text and 72 pt title; use bullet points not continuous prose; limit to one to two key figures; maximum two to three colours; leave white space between sections; consistent layout guiding the eye from title to conclusions.
30. Report findings accurately and without distortion; declare all conflicts of interest; report null results as well as positive ones; only issue press releases for peer-reviewed published findings; correct media misrepresentations promptly.

Long-Answer Questions

23. Title: concise, specific; identifies organism, IV, and DV. Abstract: 150 to 250 words; written last; aim, methods, key results with values, conclusion. Introduction: funnel structure (broad to specific); literature review; gap in knowledge; aim; hypothesis; present tense for facts, past tense for this study. Materials and methods: past tense, passive voice, third person; organisms and materials (source, condition); equipment and reagents; experimental design (treatments, controls, replication); measurement procedure; statistical analysis. Results: past tense; objective presentation; text describes main trends; figures and tables with self-explanatory captions; statistical test, statistic, degrees of freedom, p-value stated; raw data in appendix. Discussion: inverse funnel (specific to broad); hypothesis outcome stated and explained; biological mechanisms; comparison with published literature (citations); limitations; future directions. Conclusion: three to five sentences; answers research question; states significance and key limitation. References: full citation for every source cited; no uncited entries.
24. Writing style: clear, precise, objective; passive voice in methods; past tense for experiment; present tense for facts; SI units; numerals for numbers with units; species names italicised; abbreviations defined at first use. APA: author-date in-text (Author, Year); two authors (Smith & Jones, 2020); three or more (Smith et al., 2019); reference list alphabetical; journal article: Author, A. A. (Year). Title. Journal, Volume(Issue), pages. Vancouver: numbered citations in order of first appearance; reference list in citation order; journal article: Author AA. Title. Abbrev J. Year;Volume(Issue):pages. Plagiarism: presenting another's work as own; forms: verbatim copying, paraphrase without citation, data fabrication, data falsification, self-plagiarism. Avoiding it: cite all sources; paraphrase in own words; use plagiarism detection software; declare all sources in notes; do not share assignment files.
25. Oral presentation structure: title slide; introduction (background, aim, hypothesis); methods (design and key procedure); results (one finding per slide; figures preferred); discussion (interpretation, literature, limitations); conclusion (take-home message); acknowledgements; references. Slide design: plain consistent background; 6x6 rule (6 lines, 6 words per line); minimum 24 pt body, 32 pt headings; high contrast; no distracting animations; all text legible from back of room. Delivery: face audience; eye contact; clear moderate pace; pause for emphasis; handle questions honestly. Poster: A0 size; title bar; columns (Introduction, Methods, Results, Conclusions); footer; bullet points; minimum 24 pt; one to two figures; two to three colours; white space; QR code for additional data. Non-specialist communication: plain language; analogies; lead with relevance; avoid overstating certainty; use visuals. Ethics: report accurately;

declare conflicts of interest; report null results; issue press releases only for published findings; correct misrepresentations; obtain informed consent for human research; comply with national ethics guidelines.

Answers to Pilot Questions [Series 5]

Part 5.1

21. The aim of the study; the methods used (briefly); the key results with numerical values; and the main conclusion drawn from the results.
22. The funnel structure moves from broad background (the general topic and relevant published literature) to progressively more specific content (the gap in knowledge that the study addresses), ending with the specific aim and hypothesis of the study. The final sentence should state the hypothesis being tested.
23. The organisms or materials used (species, source, age, condition); the equipment and reagents (manufacturer, concentration, grade); the experimental design (treatments, controls, number of replicates); the measurement procedure (how the DV was measured, instrument, units); and the statistical analysis applied.
24. The results section presents data objectively without interpretation; it contains text descriptions of the main trends, figures and tables with captions, and statistical outcomes (test, statistic, p-value). The discussion interprets the results: it states whether the hypothesis was supported, explains the biological mechanisms, compares findings with published literature, and discusses limitations and future directions.
25. Every source cited in the text must have a corresponding full entry in the reference list, and every entry in the reference list must correspond to a citation in the text; there must be no uncited entries and no uncorroborated citations. The in-text citation (e.g. Smith, 2020) is a pointer that directs the reader to the full bibliographic details in the reference list.

Part 5.2

21. Use clear, direct language; use passive voice in the methods section; use past tense for experimental actions and present tense for established facts; use SI units throughout; write numbers with units as numerals (e.g. 5 mL); write species names in italics; define all abbreviations at first use; avoid colloquial language and unsubstantiated claims.
22. (Smith et al., 2022).
23. Author, A. A., & Author, B. B. (Year). Title of article in sentence case. Name of Journal in Italics, Volume(Issue), first page to last page. https://doi.org/xxxxx
24. Plagiarism: presenting another person's words, ideas, data, or images as your own without acknowledgement. Three forms: (1) verbatim copying without quotation marks and citation; (2) paraphrasing another's ideas without citation; (3) data fabrication (inventing data that were never collected).
25. Data fabrication: inventing data that were never collected; the fabricated numbers have no basis in any real experiment. Data falsification: selectively altering real data (e.g. removing inconvenient outliers or changing values) to produce a desired result. Both are serious forms of research misconduct that can result in retraction of publications, loss of funding, dismissal from employment, and in severe cases, criminal prosecution.

Part 5.3

21. Title slide; introduction (background, aim, hypothesis; 1 to 2 slides); methods (design and key procedure; 1 to 2 slides); results (one finding per slide; 3 to 5 slides); discussion (interpretation, comparison with literature, limitations; 1 to 2 slides); conclusion (take-home message, future directions; 1 slide); acknowledgements; references.
22. Plain consistent background (light with dark text); maximum 6x6 text density (six lines, six words per line); minimum 24 pt body text and 32 pt headings; high-contrast colour combinations; no distracting animations or clip art; all figures and text readable from the back of the room.
23. The 6x6 rule states that each slide should contain a maximum of six lines of text and each line should contain a maximum of six words. This keeps slides uncluttered and prevents the audience from reading instead of listening to the speaker; it forces the presenter to summarise rather than write out the full narrative on the slide.

24. Speak clearly at a moderate pace; make eye contact with different parts of the audience (not the screen); pause after important points to allow the audience to absorb the information; vary pitch and emphasis to maintain engagement; rehearse to stay within the allocated time.
25. Listen to the full question before responding; rephrase or repeat it for the whole audience; acknowledge that it is a good question; then say honestly that you do not know the answer but will find out and follow up (providing an email address or contact if appropriate). Never attempt to invent an answer or argue with the questioner.

Part 5.4

21. Readable from one metre (minimum 24 pt body text, 72 pt title); use bullet points rather than continuous prose; limit to one to two key figures (high resolution); maximum two to three colours; leave white space between sections; consistent layout guiding the eye logically from title to conclusions; include a QR code for supplementary material.
22. Specialist: technical jargon is used and assumed to be understood; detailed methods and statistical analyses presented; significance assessed against disciplinary standards. Non-specialist: jargon replaced with plain language; abstract concepts explained through analogies and everyday examples; lead with relevance to daily life before explaining mechanism; visuals (infographics, photographs) replace text where possible.
23. Communicating results of agricultural research (new crop varieties, fertiliser recommendations) to smallholder farmers who make planting decisions based on available information; explaining vaccine safety data to communities with vaccine hesitancy to support public health interventions and reduce preventable disease outbreaks.
24. Publication bias occurs when only positive or statistically significant results are published; null and negative results are suppressed. Consequences: the scientific literature overstates the effectiveness of treatments and interventions; meta-analyses and systematic reviews overestimate effect sizes; policy and clinical decisions are based on a distorted evidence base; resources are wasted repeating experiments that have already been done but never reported.
25. Report findings accurately and without distortion (no selective reporting); declare all conflicts of interest (financial, personal, or institutional) in publications and presentations; report null results alongside positive results to contribute a complete and honest evidence base to the scientific literature.

References and Attributions [Series 5]

Textual References (Books and External Sources)

- American Psychological Association. (2020). Publication manual of the American Psychological Association (7th ed.). APA.
- Day, R. A., & Gastel, B. (2012). How to write and publish a scientific paper (7th ed.). Cambridge University Press.
- McMillan, V. E. (2012). Writing papers in the biological sciences (5th ed.). Bedford/St. Martin's.
- Nicol, A. A. M., & Pexman, P. M. (2010). Displaying your findings: A practical guide for creating figures, posters, and presentations (6th ed.). APA.

Figures, Plates, Tables, and Boxes

- Box 5.1: Summary of the sections of a scientific report with purpose, tense, and key content Attribution: AI generated. Suggested OER search string: "scientific report structure sections abstract introduction methods results discussion conclusion references table OER".
- Figure 5.1: Annotated layout of a correctly designed scientific conference poster Attribution: AI generated. Suggested OER search string: "scientific poster design layout annotated example conference A0 columns title results OER".